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A COMPARISON OF TOTAL SERUM PROTEIN AND ALBUMIN VALUES AS DETERMINED BY THE MICRO-KJELDAHL, BIURET AND FOLIN METHODS*

By SISTER MARY WILLIAM (SALLER), MT (ASCP)
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In this study protein values of human blood serum were compared and evaluated using the three colorimetric methods commonly employed in routine analyses in clinical laboratories: the micro-Kjeldahl, the Biuret and the Folin. The efficiency of various chemical precipitants in partitioning the serum proteins was likewise evaluated. The investigation was designed to establish a rapid, simple and accurate colorimetric method for routine use in the hospital clinical laboratory for the estimation of total blood serum protein, and albumin which would give values comparable to those obtained by the electrophoretic method.

I. Historical Introduction

The basic principle underlying nitrogen determination in inorganic substances had been recorded early in the history of analytical chemistry, but its application to physiological chemistry was first recognized as possible when Kjeldahl[1] in 1883, published the results of experiments conducted over a period of time. He had observed that an organic substance when heated with sulfuric acid for a length of time would become soluble; and if this solution were then treated with anhydrous, pulverized potassium permanganate while still hot, all the nitrogen present would be converted to ammonia. The nitrogen content of the ammonia, after alkalinizing the solution, could then be determined by distillation and titration.

This procedure was subjected to detailed investigation by numerous workers[2] with the result that modifications were proposed affecting primarily: the use of catalysts, the digesting apparatus, and nesslerizing the ammonia formed, as Nessler's solution had been used for some time in analyzing the ammonia content of water.

* HILLKOWITZ AWARD, 1954. Read before ASMT convention, June 1954, Miami Beach, Florida.

A notable contribution was made by Gunning[3] who introduced the use of potassium sulfate as a catalyst which reduced the period of digestion to several hours in contrast to the original twelve hours, and eliminated a possible source of error caused by the loss of ammonia in a more prolonged digestion. Copper sulfate added to the sulfuric acid-potassium sulfate digestion mixture was favored by Folin and Farmer,[4] Cullen and Van Slyke,[5] and continued to be used by Folin and Denis[6] with the new sulfuric acid-phosphoric acid digestion mixture tested by them. As a result of these repeated analyses, the digestion period was reduced from a variable number of hours to ten minutes or less.

Wong[7,8] proved by comparative analyses that potassium persulfate was an effective component of the digestion-oxidation reaction. Digestion could be completed in approximately three minutes; the rapidity of the reaction decreasing the ammonia loss. However, Koch and McMeekin[9] maintained that the addition of a few drops of 30% hydrogen peroxide "to a solution of organic matter in concentrated sulfuric acid caused a very rapid oxidation with complete retention of the nitrogen as ammonia." This was the most rapid method yet reported, and comparative evaluation of protein substances using this method and the macro-Kjeldahl-Gunning method requiring several hours for digestion gave results which closely approximated previously determined values.

Micro-Kjeldahl methods were formulated by Folin and associates[10] because of the inherent limitations encountered in the analysis of biological fluids such as blood, urine, etc., for protein content. Consequently, the original complicated digestion apparatus of Kjeldahl consisting of flasks, condensers, water traps, and distilling apparatus[2] was replaced by a simple Pyrex test tube of 75 ml. capacity in which all the steps of the micro-Kjeldahl procedure were carried out. After repeated trials with the proportionate amounts of sodium hydroxide and Nessler's solution, they succeeded in obtaining an optically clear, precipitate free, colored compound when the reagent was added directly to the cooled solution in the digestion tube. This direct nesslerization precluded the more time consuming distillation and titration of the previous methods and made possible a colorimetric method for protein determination by the Kjeldahl method. A solution of ammonium sulfate with known nitrogen content was used as the standard for comparison.

Folin and Denis[11] in 1912, introduced another colorimetric method for the determination of serum proteins using their phenol reagent, attributing the color reactions of the proteins in solution as being due largely to the tyrosine they contained. Hence, tyrosine in definite and specific amounts was used as a standard for estimating protein content. However, various investigators maintained that tryptophane and its decomposition

products gave the same results with the phosphomolybdic-tungstic acid reagent thus yielding results too high if the tyrosine content is the criterion.

Tryptophane, and its decomposition products, as sources of error when using the phenol reagent to determine protein content were verified by Gortner and Holm[12] and substantiated by Folin and Looney[13] in a series of experiments with individual amino acids obtained in pure condition: tyrosine, tryptophane, alanine, phenylalanine, lysine, aspartic acid, histidine, glutamic acid, proline, leucine, valine, glycine, aspargine, cystine, isoleucine, isoserine and arginine. Of the seventeen amino acids tested with the phenol reagent only tyrosine and tryptophane gave a color reaction. The error contributed by indole was negligible and was therefore disregarded.

Since tryptophane manifested a comparatively slow color development with Folin's phenol reagent, and proved sensitive to alkali, Wu[14,15] adapted this method to plasma proteins. He emphasized the accuracy and simplicity of the method as compared with the procedures then in use. However, the instability of the reagent, which decomposed in alkaline solutions, and which reacted with tyrosine only when the solution was sufficiently alkaline to destroy the reagent with great rapidity offered limitations not suspected heretofore. Thus large amounts of reagent were needed to obtain maximum color development from a definite amount of tyrosine, which reaction resulted in precipitates and turbidities with an attending slow color development.

Folin and Ciocalteau[16] eliminated the turbidity factor, represented by insoluble sodium salts, with the addition of lithium sulfate to the phenol reagent.

In 1929, Greenberg[17] further simplified the method and technics of Wu. He employed the improved phenol reagent of Folin and Ciocalteau and reported that larger amounts of the reagent together with 5 Normal Sodium Hydroxide could be used without an accompanying turbidity; that by thus increasing the amount of the reagent a consequent rapid and more intense color development occurred. But, Tuchman[18] after a comparative study of the Wu and the Kjeldahl methods, was of the opinion that protein values obtained using the phenol reagent were too high.

Modification for this method was suggested by Andersch and Gibson[19] following repeated observations on the effect that heating the blood proteins in the alkaline medium had upon the color. After reinvestigating the problem, Greenberg[20] admitted the increased chromogenic value of the blood proteins, but found that the tyrosine conversion factors varied from those of Andersch and co-worker thus rendering the results questionable.

The validity of the earlier method of Greenberg was confirmed by Minot and associate[21] who found in determining the concen-

tration of tyrosine in serum protein that the values they obtained bore a constant relationship to the amount of protein present and that parallel determinations on the same sera by the micro-Kjeldahl method showed close agreement. A similar study by Wokes and Still[22] gave variable results.

The purple colored complex formed by protein in an alkaline medium upon the addition of a copper salt was the basis for a colorimetric method instituted by Riegler[23] who was the first to make use of the biuret reaction, as this phenomenon was called. He had used it in the estimation of protein content of urine but had suggested the possibility of its use in the analysis of other biological fluids. Subsequently, Autenrieth[24] extended the method for evaluating protein content by the Biuret method to ascitic fluid and blood serum. Complicating factors presented by turbidity due to the precipitation of cupric hydroxide, and a suitable standard for comparison negated the use of the biuret reaction as an accurate and reliable method for serum protein determinations.

Pure biuret was recommended by Hiller[25] as a standard, and in the work of Fine[26] serum protein preserved in chloroform provided a satisfactory standard. However, the need for a standard was eliminated when Kingsley[27] included the photoelectric colorimeter in the modified Biuret method proposed by him. In this instance, standardization was accomplished by determining the protein and albumin content of pooled human sera using the micro-Kjeldahl method. The readings obtained by the Biuret method were then plotted against the Kjeldahl values.

The relative amounts of sodium hydroxide and copper sulfate necessary to obtain the optically clear solution required for colorimetry and at the same time assure maximum color development, led Fine, Robinson and Hogden,[28] and Sizer[29] to precipitate the proteins with trichloroacetic acid; then by dissolving the precipitate with the alkali prior to the addition of copper sulfate it was shown that an optically clear solution was possible, but unstable. Turbidity in the biuret complex remained a variable factor despite the modifications of the Kingsley method.

Further investigations conducted by Kingsley[30] pertinent to the Biuret method resulted in a "one piece reagent" which could be added directly to the protein or protein dilutions and would prevent the precipitation of cupric hydroxide. Mehl[31] reported reliable values with the one piece reagent which had been stabilized with ethylene gylcol. But, Weichselbaum[32] offered significant data with the stable one piece reagent compounded by him which formed a relatively permanent, "optically clear biuret complex which obeyed the Beer-Lambert Law." Sodium-potassium tartrate was the effective stabilizing agent. His findings were duplicated and confirmed by Gleiss and Hinsberg.[33]

Modifications of Weichselbaum's one piece reagent were made by Gornall and co-workers[34] after thorough examination of the factors affecting sensitivity, stability, and practicability. By decreasing the concentration of copper sulfate and increasing that of the sodium hydroxide while maintaining a 4:1 ratio of tartrate and copper, the new reagent excluded the use of potassium iodide and effected maximum color development at room temperature. The observed differences in comparative protein analyses of the same sera by the micro-Kjeldahl and Biuret methods using the modified one piece reagent were considered statistically insignificant.

For more than a century, differentiation and characterization of the proteins constituting the blood serum has had the interests and endeavors of chemists. It was recognized that the plasma proteins presented a complex system of protein components; that certain of these were soluble in natural salt solutions and not in water and classified as globulins; that others were water soluble albumins.

The physical property of solubility manifested by the blood serum proteins served as the basis for the salt precipitation methods for their fractionation. The salts most generally used were: ammonium, sodium or magnesium sulfate, and various phosphates chiefly those of sodium and potassium.

Howe[35] was the first to establish sodium sulfate as a precipitant for serum proteins, having been influenced by the disadvantages experienced with the use of ammonium sulfate and magnesium sulfate-removal of nitrogen prior to analysis of the fraction, with the former, and the gelatinous character of the precipitate of the latter.

The data obtained by Howe with the use of sodium sulfate indicated that: increasing amounts of proteins were extracted from serum at 37° C. which appeared as a function of increased salt concentration; complete precipitation of all globulins was realized with a 22% concentration of anhydrous sodium sulfate; the total globulin components were euglobulin, Pseudoglobulin I and Pseudoglobulin II. Subsequent electrophoretic studies conducted by Cohn, and associates,[36] Taylor and Keys[37] patterned the albumin fraction obtained with sodium sulfate as albumin and alpha globulin.

In an attempt to modify the twelve hour precipitation period at 37° C. as prescribed by Howe without decreasing the amount of globulin precipitated, Greenberg[17] obtained reproducible and comparative results after two hours standing followed by filtration at the same temperature. Fine[26] filtered the mixture immediately, refiltering as soon as the filtrate cleared; and affirmed that the albumin values using this procedure showed no appreciable variation from predetermined values. Ardry[38] in a study

comparing the effect of partioning the serum proteins with sodium sulfate and sodium sulfite favored the twelve hour precipitation period for both salts.

Loss of albumin due to adsorption by filter paper as a cumulative source of error in the process of fractionation was considered at length by Goettsch and Kendall,[39] Robinson, Price, and Hogden,[40] particularly the latter group who emphasized the importance of size and kind of filter paper and the amount of solution filtered. In a comprehensive study of albumin loss through adsorption, Harris[41] determined a statistically evaluated loss of 0.05gm%.

The angle centrifuge appeared to be a solution to the problem of adsorption. Robinson, and co-workers[42] listed it as a reliable step in the technic of fractionation; clear solutions were not possible with an ordinary centrifuge. The albumin concentration of the centrifugate was consistent with previously determined values. However, limitations with this method were noted by Kingsley[43] who proposed a rapid centrifugal method for globulin precipitation. The addition of a small quantity of ether to the serum and salt mixture before centrifuging would cause the globulin to separate in a compact layer interposed between the ether phase and the clear albumin solution. Complete precipitation of the serum globulins was effected with 23% sodium sulfate.

Sulfites as serum globulin precipitants were first introduced by Campbell and Hanna[44] after detailed and comparative studies. A concentration of 21% sodium sulfite was found to be the most effective. Cohn and Wolfson[45] claimed that complete precipitation of the globulins was possible only with a 28% solution of sodium sulfite. This was confirmed by electrophoretic studies, and at the same time it was shown that alpha globulin was a constituent of the albumin fraction when a 21% solution of sodium sulfite was used. A modification[46] of their earlier method which had included immediate filtration was prompted by the ether extraction technic of Kingsley. A surface-active agent (Span 20) was included in the ether-protein-salt-mixture before centrifuging. Albumin values remained consistent.

Buffered methanol as an agent for fractionation of serum proteins at a temperature of zero degrees Centigrade was reported for the first time by Pillemer and Hutchinson.[47] Comparative electrophoretic studies attested that a true albumin fraction was obtained. Jager, et al[48] reported unsatisfactory results in a comparative study of the electrophoretic pattern and methanol as the chemical precipitant. However, Cohn and Wolfson[49] after comparative studies likewise using electrophoresis as their standard concluded that methanol was capable of precipitating the total globulin fraction without denaturing the protein.

II. Experimental

A. Total Protein

1. Micro-Kjeldahl Method

Reagents

0.9 per cent sodium chloride.
10 per cent sodium tungstate.
30 per cent hydrogen peroxide.
1:1 sulfuric acid, digestion mixture.
N/12 sulfuric acid.
Nessler's Reagent.
Standard Ammonium sulfate. 0.236 grams ammonium sulfate is dissolved and diluted to volume with distilled water in a 500 ml. volumetric flask to which a few drops of concentrated sulfuric acid are added; the nitrogen content per ml. is 0.1mg.

Procedure

One ml. of a 1:50 dilution of blood serum, the diluent used being sodium chloride, is placed in a large pyrex test tube of 75 ml. capacity which is graduated at levels of 35 ml. and 50 ml. To this is added 1 ml. of the acid digestion mixture, 1:1 sulfuric acid, and three glass beads. The contents of the tube are digested over a micro burner until the excess water has been driven off, the solution darkens, and white fumes appear. When charring is complete, two drops of 30 per cent hydrogen peroxide are added directly to the solution while still hot. The mixture decolorizes immediately, after which the flame is removed and the contents of the tube allowed to cool. Dilution with distilled water is made to the 35 ml. level followed immediately with 15 ml. of Nessler's solution. This is added rapidly and directly to the center of the solution to prevent increase of protein values resulting from the addition of Nessler's solution along the inner side of the tube, or a prolonged addition. The contents of the tube are thoroughly mixed by inversion and let stand for ten minutes to insure full color development.

A nitrogen standard is prepared at the same time with 2 ml. ammonium sulfate solution, 1 ml. acid digestion mixture, 32 ml. distilled water and 15 ml. Nessler's solution. The same precaution is observed with the standard.

After ten minutes aliquots of the solution are transferred to absorption cells and the colors read in a Klett-Summerson colorimeter with a 540 mu, green filter.

A separate analysis of the serum is made for non-protein nitrogen content. The protein of the serum is precipitated with 8 ml. of N/12 sulfuric acid added to 1 ml. of the serum and 1 ml. sodium tungstate solution. Five ml. of the filtrate are analyzed following the same digestion-oxidation reaction outlined for nitrogen determination.

The protein value of the serum is calculated using the factor 6.25 recommended by Wu[10] after correction is made for the non-protein nitrogen content. Results obtained with the sera analyzed by this method are found in Table I.

2. Biuret Method

Reagents

0.85 per cent sodium chloride.

Biuret Reagent. 2.50 grams copper sulfate ($CuSO_4 \cdot 5HOH$) are dissolved in approximately 250 ml. distilled water; 10.0 grams sodium-potassium tartrate and 350 ml. of 2.5 N sodium hydroxide, carbonate free, are added. When the solution has cooled, dilution is made to volume with distilled water in a one liter volumetric flask. The reagent is kept in a paraffin lined bottle, or one made of polyethylene, to prevent auto-reduction.

Cobalt Standard. 10.80 grams of anhydrous cobaltous sulfate or 19.59 grams hydrated salt are dissolved in 300 ml. distilled water in a 500 ml. volumetric flask to which one ml. of concentrated sulfuric acid has been added; dilution is made to volume with distilled water.

Procedure

Two tenths of clear serum are placed in a test tube of 15 ml. capacity, 1.8 ml. of sodium chloride are added and mixed by lateral shaking. Eight ml. of Biuret reagent are pipetted into the diluted serum and immediately mixed by inverting the tube several times, then allowed to stand at room temperature for thirty minutes for maximum color development.

The standard solution is used as such and requires no dilution or chemical treatment. A blank solution consisting of 1 ml. of 23 per cent sodium sulfate solution and 4 ml. of Biuret reagent is prepared simultaneously with the serum test solution.

A five ml. sample of the standard and an equal amount of the solution under examination are transferred to absorption cells after thirty minutes and read in the colorimeter after it had been adjusted with the blank set at zero reading. A green, 540 mu, filter is used.

The protein content of the serum is determined by multiplying the colorimeter readings for standard and serum by the factor 7.2 which was proposed by M. Rubin, Ph.D., of the Department of Chemistry, Graduate School, Georgetown University, Washington, D. C. This factor is the result of his research with the biuret reaction for the estimation of serum proteins, and is based upon determinations made with a commercial preparation of normal serum albumin containing five grams per twenty ml. of buffered diluent. Serum protein values by this method are found in Table I.

TABLE I. Total Protein Values of Human Sera as Determined by the Micro-Kjeldahl, Biuret and Folin Methods.

	Kjeldahl (GM %)	Biuret (GM %)	Folin (GM %)
1.	7.45	7.71	7.40
2.	7.62	7.94	6.89
3.	7.11	7.20	6.99
4.	7.88	7.48	7.80
5.	7.61	7.37	6.99
6.	7.66	7.03	7.13
7.	7.58	7.60	6.64
8.	7.60	7.21	7.37
9.	7.86	6.17	6.64
10.	6.38	6.92	7.75
11.	6.35	7.50	6.70
12.	7.35	6.50	6.15
13.	7.96	8.06	7.39
14.	7.23	8.13	7.87
15.	7.29	7.20	6.89
16.	7.27	6.92	6.64
17.	6.95	7.47	7.13
18.	6.61	7.47	8.12
19.	6.90	7.75	7.13
20.	6.92	7.75	7.13
Average	7.29 ±0.46	7.37 ±0.50	7.14 ±0.49

3. Folin Method

Reagents

5 N sodium hydroxide.

Phenol Reagent, Folin and Ciocalteau modification.

Tyrosine Standard. 50 mg. pure Tyrosine in 250 ml. of 0.1 N hydrochloric acid.

Procedure

Approximately 25 ml. distilled water are placed into a 50 ml. volumetric flask to which is added 0.2 ml. of clear serum. In quick succession 2 ml. sodium hydroxide and 3 ml. phenol reagent are added with continuous mixing. Any appreciable delay at this point will result in either a too intense color reaction or turbidity. Dilution to volume is made with distilled water; the contents of the flask are thoroughly mixed by inverting a number of times, then let to stand for ten minutes to insure maximum color development.

A standard is prepared at the same time and is treated in the same manner as the serum being analyzed. Five ml. of the tyrosine standard are used.

Readings of both standard and the solution being examined are made after ten minutes; a blue, 420mu, filter is used in the colorimeter which is adjusted to zero reading with distilled water as the blank.

To determine the protein value of the serum analyzed, the colorimeter readings are multiplied by the factor 8.0 previously determined by Greenberg.[17] Values for total protein obtained by this method are found in Table I.

B. Albumin

1. Fractionation With 22 Per Cent Sodium Sulfate

Reagents

22 per cent sodium sulfate, anhydrous (W/V). Solution is kept at 37° C.

Procedure

Into a large tube, 20 ml. capacity, is measured accurately 0.5 ml. of clear serum and 9.5 ml. of the sodium sulfate solution. The tube is stoppered and inverted several times avoiding vigorous shaking, then placed into a 37° C. incubator for two hours. The precipitated globulins are filtered at the same temperature; a retentive filter, Whatman No. 42, diameter 7 cm. is used; and the first several ml. refiltered to obtain a uniform filtrate, as recommended by Greenberg.[17]

A one ml. aliquot is transferred to a digestion tube, 1 ml. of digestion mixture is added and the nitrogen content determined by the micro-Kjeldahl method as previously outlined. The albumin value is obtained with the nitrogen conversion factor 6.25 as indicated before. Correction is made for the non-protein nitrogen content. The results as determined by this method are found in Table II.

TABLE II. Albumin Values of Human Sera as Determined by the Micro-Kjeldahl Method Following Fractionation.

	KJELDAHL			
	22% Na_2SO_4	23% Na_2SO_4	28% Na_2SO_3	Methanol
1.	3.21	3.23	3.65	3.98
2.	2.73	2.81	3.17	3.06
3.	4.43	3.29	3.84	3.98
4.	4.18	4.28	3.56	3.90
5.	4.33	3.33	4.26	4.21
6.	4.78	3.51	3.28	3.91
7.	4.02	3.77	3.52	4.42
8.	4.78	4.54	4.29	4.29
9.	3.58	3.71	2.91	3.11
10.	3.79	3.53	3.72	3.26
11.	4.04	3.26	3.17	3.54
12.	2.96	3.47	2.87	2.96
13.	4.12	4.12	3.51	3.71
14.	4.08	4.62	4.00	3.83
15.	4.21	4.42	4.30	4.37
16.	4.64	4.40	4.35	4.07
17.	4.95	4.43	4.43	3.72
18.	4.04	4.17	3.86	3.55
19.	4.41	3.86	3.86	3.42
20.	4.20	3.99	3.99	4.32
Average	4.07 ±0.59	3.83 ±0.52	3.72 ±0.48	3.78 ±0.45

The albumin content is likewise estimated on a 2 ml. aliquot according to the Biuret method. This is treated in the same manner as for protein determination. The conversion factor 7.2 is used to obtain the albumin value; these values are found in Table III.

TABLE III. Albumin Values of Human Sera as Determined by the Biuret Method Following Fractionation.

	BIURET			
	22% Na_2SO_4	23% Na_2SO_4	28% Na_2SO_3	Methanol
1	3.60	3.74	3.17	3.31
2	3.88	3.45	3.16	3.45
3	4.92	4.89	4.32	4.32
4	4.60	5.04	4.46	4.32
5	4.02	4.89	4.17	5.16
6	4.68	5.04	4.32	4.46
7	5.16	5.32	4.89	4.46
8	4.60	4.89	4.32	4.75
9	4.60	5.18	4.75	4.75
10	4.68	5.18	4.60	5.04
11	4.59	4.44	3.97	4.03
12	3.72	3.32	3.15	4.07
13	4.03	3.90	3.45	3.87
14	4.32	4.46	3.87	4.32
15	4.02	4.32	4.20	4.83
16	3.96	4.23	3.76	3.93
17	4.88	4.89	4.17	4.78
18	3.86	4.52	4.07	4.98
19	3.28	3.74	3.01	3.27
20	4.86	4.95	4.56	3.95
Average	4.31 ±0.51	4.51 ±0.60	4.01 ±0.57	4.35 ±0.57

For the albumin evaluation by the Folin Method, a 5 ml. aliquot is used. The same procedure is followed as for serum protein according to this method. In this instance, the conversion factor 6.64 is used; the factor as determined by Greenberg.[17] The results are found in Table IV.

TABLE IV. Albumin Values of Human Sera as Determined by the Folin Method Following Fractionation.

	FOLIN		
	22% Na_2SO_4	23% Na_2SO_4	Methanol
1	4.37	4.37	3.37
2	3.47	3.77	3.53
3	3.35	3.37	3.06
4	4.16	4.21	3.96
5	3.67	3.88	3.47
6	4.10	3.69	3.47
7	4.14	3.43	4.61
8	4.64	4.25	4.25
9	3.88	3.57	3.36
10	4.90	3.78	3.39
11	3.06	3.06	2.80
12	3.66	3.16	3.06
13	3.86	4.29	3.27
14	3.90	4.08	3.88
15	3.66	4.10	4.31
16	4.60	4.06	4.08
17	3.88	4.49	3.88
18	4.48	4.08	4.39
19	4.60	4.33	4.31
20	4.60	4.06	4.49
Average	4.05 ±0.49	3.91 ±0.41	3.70 ±0.53

2. Fractionation With 23 Per Cent Sodium Sulfate and Ether

Reagents

23 per cent sodium sulfate, anhydrous (W/V). Solution is kept at 37° C.

Procedure

One ml. of clear serum and 9 ml. of sodium sulfate solution are mixed in a 15 ml. centrifuge tube by inverting several times. Three ml. of ether are quickly blown into the mixture, the tube stoppered, and shaken vigorously for thirty seconds. The tube is then placed into the centrifuge which is set at 3000 r.p.m. and allowed to run for ten minutes. The globulin separates out in a compact layer between the ether phase and the clear albumin solution. To withdraw the albumin solution, the tube is held at an angle, the side tapped gently to dislodge the globulin layer, then a thin pipette is inserted beneath the ether and globulin layer.

The albumin content of 1 ml. of 1:1 aliquot is determined by the micro-Kjeldahl method; the values computed as before. These values are found in Table II.

A 2 ml. aliquot is used to determine the albumin content by the Biuret method, and 2.5 ml. aliquot, for the Folin method. The conversion factors 7.2 and 6.64 are used for the values obtained in Tables III and IV respectively.

3. Fractionation With 28 Per Cent Sodium Sulfite

Reagents

28 per cent sodium sulfite, anhydrous (W/V).

Procedure

To 18 ml. of sodium sulfite solution in a small Erlenmeyer flask are added 2 ml. clear serum dropwise; during the addition the flask is agitated gently with a swirling action. The mixture is filtered immediately through two thicknesses of Whatman No. 42 filter, diameter 7 cm.; the first ten ml. of the filtrate are discarded.

One ml. of a 1:1 dilution of a portion of the filtrate is analyzed for albumin content by the micro-Kjeldahl method, correction being made for the non-protein nitrogen content of the serum, and the nitrogen conversion factor used in the evaluation. Values obtained by this method for the sera analyzed using this specific precipitant are found in Table II.

The albumin value for a 2 ml. aliquot is determined according to the Biuret method. Table III shows the results for the sera analyzed by this method following precipitation with this salt.

The reduction of cystine to cysteine by the sulfite precipitant precluded albumin determination by the Folin method, since cysteine gives a blue color with the reagent.

4. Fractionation With Methanol

Reagents

Methanol Reagent. 607 ml. of methanol are added to 393 ml. distilled water, cooled to 0° C.; diluted to one liter with cold methanol. Solution is kept at 3° C.

Acetate buffer. 72 ml. of 1 M acetic acid and 12 ml. of 1 M sodium hydroxide are diluted to volume in a 100 ml. volumetric flask with distilled water. Solution is kept at 3° C.

Procedure

Two ml. clear serum are placed into a 20 ml. test tube; one ml. of acetate buffer is added with constant stirring which is continued as 7 ml. of methanol reagent are added. The tube is stoppered and let to stand at a temperature of 3° C. for thirty minutes. The precipitated globulins are removed by filtration using a retentive Whatman No. 42 filter paper, diameter 7 cm. Filtration is carried out at the same temperature of 3° C.

All glassware, excepting the pipettes, are chilled in the refrigerator which maintains a temperature of 3° C. at all times.

Albumin determinations are made on aliquots of the filtrate as follows: one ml. of 1:4 dilution, by the micro-Kjeldahl method as described; one ml. by the Biuret method; and 1.25 ml. according to the Folin method. The values obtained are found in Tables II, III and IV.

III. Discussion

A summary of the protein values as presented in Table I indicate that all values are within the range of normal limits as determined by the micro-Kjeldahl, Biuret and Folin methods. The mean value of the twenty sera analyzed by the micro-Kjeldahl method as shown is 7.29 gm% with a standard deviation of ± 0.46; for the Biuret method, 7.37 gm% with a standard deviation of ± 0.50; and for the Folin method, 7.14 gm% with a standard deviation of ± 0.49. The observed differences when evaluated statistically using the values obtained by the micro-Kjeldahl method as the standard for comparison can be considered of no significance.

Comparative study of the albumin values as tabulated in Table II, values determined after precipitation with the chemicals recorded and by the micro-Kjeldahl method, reveal no differences that are of significance within the method itself that are attributable to a specific fractionating agent. The same conclusion is reached for the data presented in Table IV.

Analysis of the albumin values by the Biuret method, shown in Table III, indicate that although the four means differ, the differences are not statistically significant. As a consequence any one of the four methods may be employed for albumin determination.

However, values obtained by the three methods demonstrate significant differences, particularly with reference to the albumin values obtained by the Biuret method. In a comparison of the means, using the values of micro-Kjeldahl method as the standard for comparison, the value for "*t*" in this instance, which was not significant in the previous evaluations, was found to be 5.3.

The higher values for the Biuret method are attributed to the conversion factor 7.2 used in these determinations. This factor was the result of analyses of commercial albumin, and against which the method had been standardized. If the standardization had been effected against human serum, the albumin value of which had been previously determined by the micro-Kjeldahl method, the factor would approximate the conversion factor recommended by Wu, and show consequent lower albumin values when determined by the Biuret method.

IV. Conclusion.

The total protein content of serum as determined by the three methods described show close agreement. Thus, an estimation by any one of the methods would be of equal value.

Any salt fractionation method is liable to inaccuracies because of the inherent solubility properties of the several serum proteins. At various salt concentrations there is an overlapping of solubilities; hence, true albumin fractions would be difficult to realize.

The close approximation shown by the albumin values, obtained following fractionation with the various salts, to those obtained by methanol precipitation, which is in accord with electrophoretic values, indicate that salting out, as a method of differentiation of protein components, is reliable.

From the standpoint of time, ease of manipulation, and elimination of filtration, the determination of serum albumin using the Kingsley procedure would be the method of choice; the Biuret method for protein and albumin evaluation.

V. Summary

1. A comparative evaluation of the total protein content of twenty samples of pooled human sera was made. Micro-Kjeldahl, Biuret and Folin methods were used in the analyses.

2. Fractionation of the serum protein complex was effected by chemical precipitation. The precipitants used were: 22 per cent sodium sulfate at 37° C., 23 per cent sodium sulfate and ether, 28 per cent sodium sulfite, and methanol at 3° C.

3. A comparative evaluation of albumin content was made on each filtrate resulting from the chemical precipitation. Micro-Kjeldahl, Biuret and Folin methods were used in the analyses.

4. Statistical evaluation was made on the data presented.

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THE USE OF THE ADMINISTRATIVE MANUAL IN A CLINICAL LABORATORY AND SCHOOL OF MEDICAL TECHNOLOGY*

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All authorities on the Art of Administration agree that an organization exists to accomplish certain tasks or objectives. It is the duty of those comprising the administrative section of this organization to think and plan about these organizational achievements so as to facilitate the best possible use of the co-operative efforts of a group of trained individuals. This is also true of the groups that comprise the personnel of any hospital and clinical laboratory. Without organization and forethought there will be wilful waste of talents and manhours.

In view of our conviction of the importance of administrative organization in the training of student Medical Technologists and in the efficient supervision of a Clinical Laboratory, we have complied two types of manuals: one the administrative manual and the other the procedure manual. It was also the fruit of an awareness of the need of administrative succession if an institution is to go forward. These manuals are of value to us because they facilitate a smoothly working department. Anyone stepping into the position of director or supervisor of either the laboratory or school of Medical Technology would have a knowledge of the background and the current organizational policies so that there would be a minimum of confusion and retracing the steps of progress already made by the institution.

We consider the procedure manual as a guide to the performance of a specific job. This type of manual gives detailed instructions as to the steps and tests in the Clinical Lab. On the other hand, the administrative manual formally states the policies and organizational activities of the laboratory administration.

All the material that goes to make up this type of manual must be specific, clear, inclusive and tractable. All policies of any department in the hospital should be ascertained, approved by the administration and then formally stated in writing. Although hospitals vary in many respects there are also just as many instances in which they are similar. One of the main common denominators is the need of all for formulated principles of organization in regard to the policies of the institution and the personnel of the department. A happy satisfied staff is half the battle of running an efficient department. All employees work best and

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accomplish the goals of the organization more effectively, if they have a clear understanding of what is expected of them in their respective positions and in turn what they can expect from the organization. With these convictions in mind we compiled the administrative manual for the clinical laboratory, showing first the lines of authority by means of an organizational chart. To know the proper channels of authority makes for a more co-operative department with very little friction when dealing with problems that arise. After we have clarified this point it naturally leads to the question of the purpose and scope of the laboratory. We have formulated the purposes of the laboratory and in so doing have tried to make the staff aware that they are a service department with responsibilities to the other departments of the hospital, to clinic, the interne staff, the visiting staff and, most of all, to the patient for whom the hospital exists. We think that it is well to have these responsibilities stated so that each member will know what the attitude of the department should be toward the other functions of the hospital. Hence, is established a more co-operative spirit within an institution.

At times, we are requested to complete questionnaires as to the physical features of the clinical laboratory, therefore, one of the sections in the manual deals with a general description of the department listing the number of square feet that comprise the space allotted to laboratories. For interest we have included a picture of a miniature of the laboratory. This section could also be developed by means of a floor plan.

Since it is essential that a laboratory hold to certain standards formulated by the accrediting bodies for hospitals, we have included a copy of the minimum standards for laboratories. With the formation of the new joint-commission for accreditation of hospitals it is realized that the present standards are subject to revision. In order that all technicians, who are in a supervisory capacity or who are responsible for certain sections of the department, have a clear understanding of their job analysis we have standardized a list of responsibilities of all. The job responsibilities of all other personnel such as the secretaries, clerks, aides and orderlies are also formulated. With a large staff we feel that it is absolutely necessary to have this type of understanding if things are to move smoothly. As we have stated before, it is essential that people know what is expected of them and be intrusted with their share of the responsibilities in a department.

Certain internal controls, commonly referred to as policies, form the essential principles upon which the medical technologist and secretarial help will base many of their decisions. Every organization is desirous of good public relations and this is particularly true of a hospital. There should be formally stated

policies covering all the phases of operations characteristic of that department. Policies concerning emergency work, making appointments for private out-patients, dispensary work, hours for the staff, vacations, overtime pay for night call, test to be done by the technician on night call, regulations as to the replacement of blood, admission routine, charges and records kept in the lab, are just a few of the essential items that should be definitely defined.

For the most part once a department has a systematized statement in writing of its policies and has the approval of the directors, it will have very little difficulty adhering to them. It is only fair to the Medical Technogolists to have some guide and backing. One instance in our actual experience deals with making appointments for private patients. It has been a help in distributing the work and prevents the unpleasantness that may result when a patient comes to the laboratory just before closing hours. It has ultimately added up to good public relations and has been more fair to the employees. After all, there are many occasions when emergencies come to the hospital at closing time and the personnel must stay overtime, but when this can be helped, it should be avoided. We all know that a human being can work for only a certain time at detail work without becoming excessively tired. If we do not do all in our power to organize our departments in such a way, then we are to blame when careless work is done or when serious errors are made. One thing that we try to stress about carrying out these regulations is that the patient is never to be penalized if there are any infractions of these formulated policies. It is one of the duties of the chief technician or laboratory supervisor to tactfully handle all such matters. To us, organization in the phases cited above constitutes the difference between efficiency and chaos and the difference between a happy contented staff and a disgruntled over-worked one.

Since record keeping is so detailed in a clinical laboratory every effort should be exerted to make this procedure as simple and inclusive as possible. This necessitates handling of numerous forms. Each laboratory should have specific forms for keeping records of results of laboratory tests and such items as, donors received and their blood types, and other forms for keeping daily accounts of tests performed. There should be a specific procedure about ordering and charting suited to the institution in which one is working. In order to clarify the types of forms and uses of each we have a section in the administrative manual with samples of all types of printed form in current use. It is a help in explaining the method of record keeping in use in the laboratory to persons who need this information.

In our institution the laboratory head, with the approval of the administrator, sets the charges for the tests performed. These charges are made out daily by one of the secretaries and sent to the business office. Therefore, we have included an accumulated list of costs for laboratory tests in the manual. Frequently other institutions in the city ask for a copy of these charges as a comparison; also physicians and others wish this information.

We feel every laboratory should have some type of inventory list of at least the non-expendable equipment in use. Under the heading of available facilities we have formulated a list of major pieces of equipment, their number and type.

Since the schools of Medical Technology are so important to our organization and profession, we shall attempt to discuss the Administrative manual for the School in some detail. It is important to have organization and planning in the program for training students, as well as in management of a clinical laboratory. If we are to be classed as a profession, our schools should be conducted as professional schools, that is, we should have a definite educational organization or program planned to accomplish the objectives of our school. The fact that a student from our institution passes the Registry examination does not of itself, indicate that we have an adequate training program. After all, the examinations in use, at the present, test, in main, the individual's ability to memorize facts. The very best criteria for measuring the effectiveness of our program is an evaluation of the end product or in other words, the performance of our graduates after they leave our school. Of course, we realize that such a testing program as we now have is necessary but we should not be smug about our schools unless they are organized to graduate students who are equipped to keep up the highest standards of our profession.

In compiling the administrative manual we have tried to assure an adequate teaching program and adherance to this program in training student Medical Technologists. The contents of the manual include such things as a statement of the objectives of our school. The formulating and stating of objectives is one of the fundamental steps in organizing an educational program since the purpose must be clarified so that instructional activities will meet the objectives. These stated objectives will vary with the institutions conducting the schools as they are influenced and formulated by the philosophy and aims of the institute. We have listed a four-fold objective which concerns itself with moral, medical ethics, attitude toward the ill and the development of skills. The essentials of an approved school of Medical Technology are included in the manual. The reason for this needs no explanation if we wish to be approved.

Following this we have a brief history of our school which

contains such data as the dates and persons responsible for the beginning of the Laboratory, the dates of the approval of the school and the data on the affiliation with the college as a four year school leading to a B.S. in Medical Technology. Each year items such as the aforementioned are added. This is not only of interest but we have had occasions when this information was needed.

Much of the contents of the manuel is devoted to the program. The purpose of any program is to define the area of operation, so we have done this under the headings of policies which deal with the general over-all description of the course and policies to be followed in regard to the student. Secondly, the subject curriculum is outlined in detail. The "Curriculum for Schools of Medical Technology" was used as a basis for this topical outline. Upon this we have developed units of study for each of the departments in the Laboratory. The Units, as such, are not included in the manual. These Units are kept in separate books and copies are also given to the student. They contain such things as the class hours, methods, tools, visual aids, doctors' lectures and outline of the instructors' lectures to be given. We have found it very useful to have the instructors' lectures outlined as it gives sequence to the curriculum and eliminates overlapping of the subject to be taught. Folowing the topical outline of the course we have a copy of the daily planned of each instructor which contains such information as the amount of work to be covered in a stated time, a list of the activities—demonstrations and the doctors' lectures. If there are specific assignments, these are included in the daily plan.

Besides the subject matter curriculum we have a schedule of the functional curriculum which is the internship training and departmental rotation. In the beginning of the year a schedule is made which lists the various departments in which the student is assigned for experience situation training. This phase is placed in the senior year of college to give the student experience in the actual situations and test of a clinical laboratory. Theoretical work has its place in the program but under no circumstances should the actual situation training be neglected nor should it lack planning and supervision. Neither of these types of training should be substituted for the other but a balance should exist between them to develop well trained technologists. For the curriculum to be a dynamic program there must be informational material plus experience situation set up for learning. This is one of the strongest arguments for hospital affiliation for colleges offering degrees leading to a B.S. in Medical Technology and for hospital to have an organized plan of training so that the schools are conducted as professional schools for learning and not for convenience. As we may see, hospitals who

are conducting true approved schools, are carrying an added burden and not obtaining cheap labor as is so commonly stated. They are doing a great service to our profession of Medical Technology.

The faculty's place in the program is paramount. Reams could be written about the importance of the staff in regard to the training of the student. In this manual we have listed the doctors who are responsible for the techniques and the results of each of the departments in the laboratory. The responsibility of these doctors is to see that the teaching is adequate and accurate. However, the bulk of the teaching is done by the technologist staff. In the manual we have listed the staff including records of such things as degree, registration, years of experience and teaching assignment. The teaching assignment is divided into two groups; those who are responsible for lecture hours and those responsible for experience training. All graduate personnel are encouraged to be student conscious. When they are employed it is with the understanding that teaching the student is as much a part of their jobs as performing the laboratory tests. In view of the responsibility of the graduate to the student, every effort is exerted to induce the graduate to keep ahead in her field by post-graduate work, attendance at state meetings, lectures, conventions and attending informative lectures at the hospital. In order that we have on record information as to what the graduate is doing to improve her ability, each one is asked to complete a report giving information as to the type of meeting attended, by whom conducted, subject matter discussed and the role of the graduate in this meeting. A copy of this form is added to the manual.

The statistical report for the year is included in the manual. This enables one to see the activity of the laboratory and the wide variety of experience that we are able to offer to the students in their internship. This is followed by what we call the measurement in the program or the system of marking which is used. The results of the curriculum are measured as objectively as possible. We have a uniform mark sheet that is in use for both the third year theoretical work and the internship. A copy of this sheet is on file at the hospital and a copy sent to the college. These marks are included in the photostatic copy of grades sent out to the students from the college. When the students take the National Registry examination the results are also added to the college records. Besides this type of evaluation we also send out questionnaires after the graduates have been working for 5 to 10 years asking for information as to the type of work they are doing and for suggestions that might help us improve the type of training offered. This enables us to evaluate the end product of our program. There

is a section in the manual that deals with the printed forms that are in use in the school. It contains samples of the certificate, class pin, catalogue and the registry forms, mark sheet application for entrance to the school. For anyone not familiar with these forms this is a tremendous help in explaining their use.

A list of the books which are available in the Laboratory for the use of the students is also among the items in the administrative manual. We feel that the library is an important tool in the education and preparation of both the student and the faculty. Since the students are required to do all the reading suggested by the curriculum of schools of Medical Technology we try to maintain an adequate supply of books that are strictly for the use of the laboratory personnel. Besides those listed in the manual the students and graduate personnel have access to the Doctors' library in the hospital. The amount of reading done is considered when marking the student.

Since we aim to have a well-rounded personality in the person of the graduate technologist, many activities are encouraged and one which creates a great deal of interest is the bowling team. People who can play together will work well together. In the manual there is listed a calendar of extra-curricular events at the college. All the students take part in these activities. One of the highlights of these social affairs is the dinner party given at the hospital for all the laboratory personnel in honor of the graduating class. It is always quite a formal affair at which the administrator presents the certificates to the graduates and the State president of the society speaks to the graduating class. It is generally at this occasion that the junior class receives its pins from the school and is now considered as senior student Medical Technologists. Since the beginning of the school we have graduated about 219 students.

Each year there are always slight modifications and revisions that are necessary in the manual if it is to be a working manual not a stagnate accumulation of worthless data. Having given a resumé of this manual it remains only to hope that this administrative tool will be an incentive to others to formulate an organizational guide to the needs and goals of their own institutions.

AMEBIASIS*

By H. W. BROWN, M.D., *School of Public Health*
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Endameba histolytica, the protozoan responsible for intestinal and hepatic amebiasis, was first reported from a patient in the cold climate of old St. Petersburg, Russia. Due to the prevalence of this infection in tropical areas, the disease was misnamed "tropical dysentery," which for years obscured the fact that infections with *E. histolytica* are common in the temperate zone. Stool surveys from various sections of the United States reveal infection rates varying from 2.1% of 562 medical and dental students in St. Louis to 36.4% of 374 inhabitants of rural Tennessee. The infection rate varies considerably and is usually highest in persons of the lower economic groups, due to poor sanitary habits, and in general is most common in warmer climates for the same reason. Craig, however, reported a 12.7% infection rate with this parasite among a group of physicians, indicating its wide distribution in the upper economic group.

Endameba histolytica assumes two forms in man: The trophozoite stage, which is the active feeding pathogenic stage; and the cystic stage which is the infectious form. The trophozoites of *E. histolytica* vary in size from 15 to 60 microns in diameter, with an average diameter of 25 microns. In freshly passed stools the amebae are very motile, moving along by the extrusion of pseudopodia in a slug-like manner. The nucleus is usually invisable in the living amebae, but ingested red blood cells may be observed in their endoplasm. The cysts of *E. histolytica* are spherical and vary in size from 5.0 to 20 microns in diameter. The completely developed cysts contain 4 nuclei which are not clearly seen in living specimens; red blood cells and other debris do not occur in cysts. Staining the trophozoites and cysts with iron hematoxylin brings out their nuclear structure and other morphological characteristics.

Cysts of *Endolimax nana, Giardia lamblia* and immature *Endameba coli* cysts also contain 4 nuclei and they must be differentiated from those of *E. histolytica.*

The trophozoites are found in fluid and semi-fluid stools, while the cysts are found in stools of formed consistency. Therefore, individuals suffering from amebic diarrhea or dysentery are not important sources of infection, whereas the cyst passers or chronic carriers with more or less normal bowel movements serve as sources of the infection. Man becomes infected with *E. histolytica* through the ingestion of the cysts in food, which may be contaminated by food handlers or flies and other insects,

* Read before ASMT Convention, June, 1954, Miami Beach, Florida.

through the use of human excrement for the fertilization of vegetables, and through water which may be contaminated by excreta or sewage. The cysts, when swallowed, pass unaffected through the stomach and upper part of the small intestine. In the ileum, due to the action of intestinal secretion, the cyst wall is weakened and the activated multinucleated ameba forces its way out of the cyst. Cytoplasmic division results in the production of four or eight small actively motile amebae which pass into the caecum, and through the action of their cytolytic substances they penetrate the intestinal tissues.

The trophozoites that invade the tissues multiply by binary fission and produce lesions of varying degree, which result in the protean disease, amebiasis. Some trophozoites which find their way into the lumen of the bowel develop into cysts which are passed in the feces. It is extremely unlikely that these cysts, once formed in the large intestine, excyst in the lower regions of the large intestine of the carrier host and produce a reinfection. The action of the gastric juice and secretions from the small intestines are probably necessary to produce infection by the mature cysts that are swallowed (Figure I). This fact is of

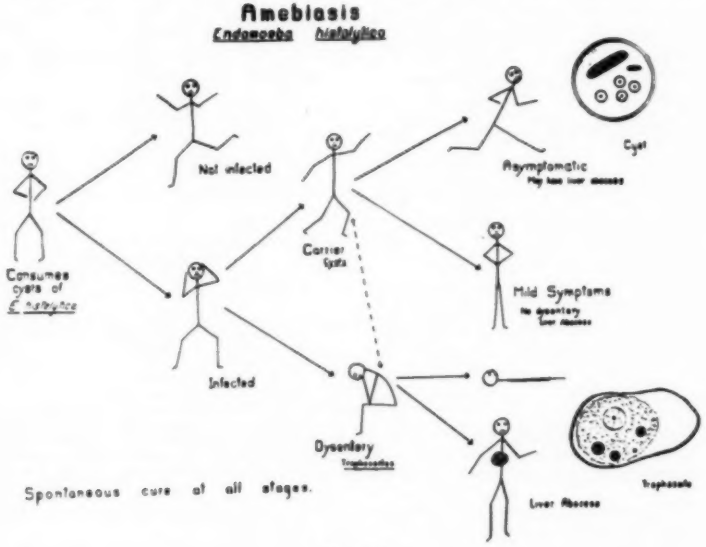

Figure I

***Endameba histolytica* cysts are ingested by man and initiate the infection of the colon. Dysenteric patients pass trophozoites whereas cysts are passed in the formed and semiformed stools of carriers.**

some importance, for it indicates that therapy need be directed against only the trophozoite stage, which fortunately is more susceptible to chemotherapy than are the most resistant cysts.

Pathology

E. histolytica is a tissue parasite and the lesions produced by it are the result of its cytolytic secretion and the penetration of tissues through its ameboid movements. The lesions produced by *E. histolytica* in the large intestine vary greatly in extent and severity. The early lesions are superficial areas of mucosal necrosis leading to minute flask-shaped ulcers. These lesions may become enlarged into extensive crateriform ulcers with ragged edges which later intercommunicate through sinuses. Secondary bacterial infection may complicate the pathological and clinical picture.

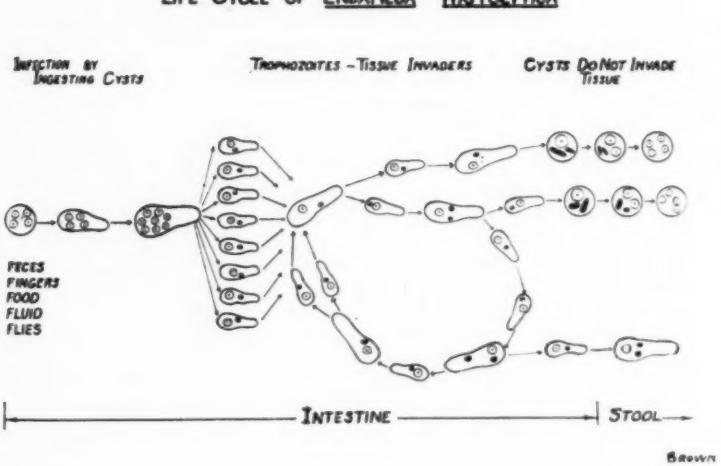

Figure II

Walker and Sellards experimentally infected man with *E. histolytica* and demonstrated that some became asymptomatic carriers, some had mild symptoms, others severe dysentery, while some individuals were not susceptible to infection.

The complement fixation test as reported by Hussey and Brown does not detect the intestinal infections but is specific for hepatic infections.

The complement fixation test is usually positive (80%) in hepatic amebiasis.

The complement fixation is usually negative in most diseases of the liver as well as other diseases. However, an occasional false negative result is encountered.

The classical studies of Walker and Sellards (1913) demonstrated the various courses that infection with *E. histolytica* may take (Figure II). These workers fed cysts of this parasite to 20 volunteers, producing infection in 18 but were unable to infect 2 individuals in spite of repeated attempts. Fourteen of those infected became carriers and passed cysts in their stools. The so-called carrier may be symptomless or have gradations of diarrhea alternating with constipation, abdominal pain (with or without tenderness), nausea, anorexia, pains in leg and back, headache and what might be termed general malaise. A carrier may eliminate his infection spontaneously or he may develop dysentery. Only 4 of the 20 individuals fed the cysts of *E. histolytica* by Walker and Sellards came down with dysentery after incubation periods ranging from 20 to 95 days. Amebic dysentery varies greatly in different individuals and the onset may occur suddenly or insidiously. The stools may number from 8 to 40 in 24 hours and their consistency range from semiformed to blood-stained mucus with shreds of necrotic intestinal mucous membrane. Abdominal and lumbar pain, nausea, vomiting and fever may be present and the patient may be prostrated.

The intestinal infection with *E. histolytica* may metastasize by way of the blood stream and produce amebic hepatitis, pulmonary and brain abscesses. Amebic hepatitis which may develop into a liver abscess is characterized by enlargement and tenderness of the liver, fever and neutrophilic leukocytosis. Amebic liver abscess is most commonly located in the right lobe of the liver. The symptoms noted above are intensified and the pain in the liver area may be transmitted to the right shoulder; chilly sensations and sweating may occur with slight rigidity of the abdominal muscles and the chest or abdomen may bulge if the abscess is large and near the surface.

Laboratory Diagnosis

Clinically, intestinal amebiasis must be distinguished from bacillary dysentery, chronic ulcerative colitis, bacterial diarrheas, food allergy, schistosomal and balantidial dysentery.

The diagnosis of infection with *E. histolytica* depends upon the demonstration of the parasite in the feces or tissues. A complete stool should be obtained, for in addition to fecal material it may contain blood, mucus and sloughed tissue debris. The flecks of mucus may contain active trophozoites. Formed and semiformed stools usually contain only the cysts of *E. histolytica,* whereas diarrheal or dysenteric stools contain the active, trophozoite stage. With this in mind one can intelligently select particular parts of the stool for examination and anticipate the stage of the ameba that will be found in a positive stool.

Fecal Smear Technic

In the United States, where most of the intestinal amebiasis is of the mild or carrier type, the stools are usually semiformed or formed, and the chance of finding trophozoites is slight, as they have rounded up and formed cysts by the time the stool is evacuated. Freshly evacuated, formed stool may be kept for 24 hours at room temperature of 70 - 75° F. and for a week in a refrigerator without loss of the diagnostic characteristics of the cysts. Examination of stools for *E. histolytica* is made difficult or impossible by the presence of free oil, bismuth or barium and, therefore, stools following oil purges or X-ray studies employing barium should be avoided.

The examination of a cold stool is carried out as follows: A small drop of physiological saline is placed on a clean microscope slide, and a small amount of feces (about the size of a half a rice grain) is transferred to it with a wooden applicator. The feces is thoroughly mixed with the saline and covered with a coverslip. The resultant smear should be thin enough so that ordinary newsprint can be read through it. A second smear can be made on this same slide substituting an iodine* solution for the saline. The iodine solution stains the nuclei and chromatoidal bars, thus aiding greatly in differential diagnosis. Fifteen to 25% of the positive *E. histolytica* cases are detected by a single stool examination. The unstained smear is examined for the active trophozoites and the iodine-stained smear for protozoan cysts, which are pictured in the several textbooks of parasitology.

Concentration Technic, For Protozoan Cysts

Faust, et al (1938) originally described the zinc sulfate centrifugal flotation method. This method is useful in detecting helminth eggs as well as protozoan cysts. Protozoan trophozoites are so greatly distorted by the zinc sulfate as to be unrecognizable, hence this technic is of absolutely no value in the detection of this stage. For routine laboratory work, this technic is the method of choice for the diagnosis of most helminth and intestinal protozoa infections.

a) Prepare a fecal suspension by comminuting one part of stool (the size of a marble) in ten parts of lukewarm tap water.

b) Strain approximately 10 ml. of the suspension through one layer of wet cheese-cloth, held in a small glass funnel, into a Wassermann tube.

c) Centrifuge the suspension in the Wassermann tube for 1 minute at 2,640 r.p.m. or top speed of the International

* The iodine solution of Sapero, Lawless and Strome (1951) is made up of 10 parts Lugol's solution, 15 parts formaldehyde (USP) and 75 parts tincture of merthiolate 1: 1,000 (Lilly).

clinical centrifuge. The supernatant fluid is poured off and 2 to 3 ml. of tap water added, and the sediment completely broken up by shaking the tube or with an applicator, and additional water is added to fill up the tube. Repeat this two or three times until the supernatant fluid is clear.

d) When the last supernatant is poured off, add 3-4 ml. of the zinc sulfate solution of 1.180 specific gravity. Break up the packed sediment and add enough zinc sulfate solution to fill the tube to within ½" of the rim.

e) Centrifuge for 60 seconds at top speed.

f) Using a bacteriological loop, remove several loopfuls of the diagnostic material floating on the surface onto a clean slide. Add one drop of iodine stain, agitating the preparation to insure a thorough mixing.

g) Mount preparation with a cover glass and examine under the microscope.

Dysenteric Stools and Saline Purged Specimens for Trophozoites

The liquid stools of patients with acute amebic dysentery will contain active trophozoites, so the microscopic examination should be made immediately, before the stool cools with a resultant loss of motility of the parasite. A fleck of blood-stained feces should be placed in warm saline on a slide and examined for motile amebae under the 16 mm. objective (Figure III). Any

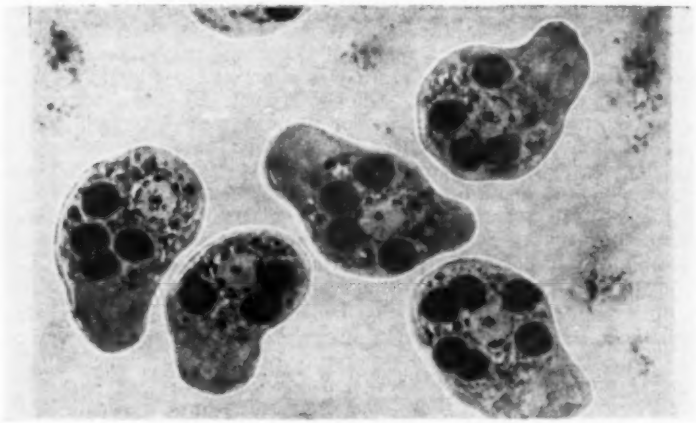

Figure III

Endamoeba histolytica **trophozoites (unstained) containing red blood cells. Note size in comparison to red cells. This is the pathogenic stage found in semi-liquid and liquid stools.**

parasites found should be studied under the 4 mm. objective; the progressive motility, hyaline finger-shaped pseudopodia and the presence of red blood cells in the cytoplasm are diagnostic of *E. histolytica*. Leukocytes are present in amebic dysentery stools, and one may find lanceolate Charcot-Leyden crystals. Examination of material from several portions of the stool or even from many stools may be necessary to detect the amebae. One must remember that bacillary dysentery is more common than amebic dysentery. Stools from bacillary dysentery patients contain numerous pus cells, and macrophages containing red blood cells may trap the unwary.

Not infrequently, repeated examinations of formed stools fail to reveal *E. histolytica* infections even though there is clinical evidence of infection. When the infection is largely confined to the cecal region, trophozoites extruded may disintegrate by the time the stool containing them is passed, which may explain their absence in the stool examination. Purgation will flush the active *E. histolytica* trophozoites out of the cecum and the whole large intestine. In our laboratory the specimen is obtained and examined as follows: The patient is told to appear at the laboratory at 9 a.m., without eating breakfast. The patient is given 30-60 cc. of a 50% magnesium sulfate solution and a chaser of water. Sodium sulfate or Phospho-Soda may be preferred by some workers, but for cheapness and assured efficacy, Epsom salts are entirely satisfactory. The patient passes his stools into a waxed carton in a toilet adjoining the laboratory and immediately returns to the laboratory with the specimen. The first feces evacuated as a result of the purge may consist of residual formed material, and this is examined for cysts by the zinc sulfate concentration method of Faust. The stool specimen is not considered satisfactory for examination for trophozoites until it is entirely liquid, for it is in material of this consistency, freshly washed out of the ulcerated areas, that one has a good chance of finding motile trophozoites. A fleck of the liquid feces is mixed with a drop of warm saline on a microscope slide and examined as above. Occasionally, when examination of the first fluid stool fails to reveal amebae, they are found on the examination of the second or third fluid movement following purgation.

One wonders, of course, what the chances are of detecting the amebic infection by the above purge procedures, and this varies with the thoroughness and experience of the technician. Magath (1934) states that his observations over a period of 15 years, resulting from more than 60,000 examinations, indicate that under proper conditions in about 80% of the cases infection with *E. histolytica* can be detected on first examination, 95% of the infections can be found by examining two specimens and approximately all the infections can be detected by three such

examinations. Shookhoff (1952) reports finding *E. histolytica* in the first diarrheal specimen in 208 out of 282 individuals (81%). If stool examinations are negative, one may wish to sigmoidoscope the patient and secure a specimen for microscopical examination from any lesions that are visualized. We have not found stool culture for amebae to be a rewarding routine procedure except in special instances where rounded up, unidentifiable amebae are found in the stool, or when the patient's signs and symptoms are highly suggestive, yet no amebae are found by microscopic examination. Difco medium is used in my laboratory and the culture is incubated for 48 hours before examining it microscopically.

Long training and considerable experience are necessary before a technician can identify the various intestinal protozoa of man. It is the most difficult diagnostic problem of the parasitologist, and months of constant experience and study are necessary to develop skill.

Recently Cole and Kent (1953) developed an *Endameba histolytica* immobilization test. Their preliminary studies indicate that serum from rabbits immunized with *E. histolytica* temporarily immobilizes the trophozoites, whereas serum from non-immunized rabbits does not. Such a test, if sensitive and accurate, would have obvious advantages.

In view of the difficulties of finding *E. histolytica* in the stool and also distinguishing it from the non-pathogenic inhabitants of man's intestine, Craig and others attempted to develop a complement fixation test which is both sensitive and accurate. If available, the advantages of such a test are obvious: large numbers could be done routinely just as the various serological tests of syphilis are done. There are scores of laboratories with technicians trained to do complement fixation tests for every laboratory that has a technician really skilled in the fecal diagnosis of *E. histolytica.* Unfortunately, the complement fixation test for intestinal amebiasas cannot be relied upon. Paulson and Andrews (1938) state, "From a practical diagnostic standpoint the too numerous falsely positive as well as falsely negative responses obtained under the conditions of our observation show the test is unreliable in the individual case." Magath and Meleney (1940) likewise found the test of questionable value and note that a considerable improvement in the preparation of antigen will be necessary before the test can be generally adopted. By contrast, Hussey and Brown (1950) and McDearman and Dunham (1952) have found the complement fixation test to be of considerable diagnostic value in hepatic amebiasis and abscess. The sera of 82% of their patients gave a positive test, and there were very few false positives. (Tables I, II and III.)

TABLE I

THE COMPLEMENT FIXATION TEST
IN INTESTINAL AMEBIASIS AND UNINFECTED INDIVIDUALS

		CASES	PER CENT
INTESTINAL AMEBIASIS	STOOLS POSITIVE	124	
	COMPLEMENT FIXATION −	−121	97.5
	COMPLEMENT FIXATION +	+ 3	2.5
NO INTESTINAL AMEBIASIS	STOOLS NEGATIVE	123	
	COMPLEMENT FIXATION −	−111	
	COMPLEMENT FIXATION +	+ 12	

Hussey & Brown

TABLE II

THE COMPLEMENT FIXATION TEST IN HEPATIC AMEBIASIS

	CASES
A. UNQUESTIONED HEPATIC AMEBIASIS	
COMPLEMENT FIXATION +	+ 10
COMPLEMENT FIXATION −	− 2
B. PROBABLE HEPATIC AMEBIASIS	
COMPLEMENT FIXATION +	+ 4
COMPLEMENT FIXATION −	− 1

Hussey & Brown

Chemotherapy

The treatment of amebiasis, for practical purposes, may be considered under the heading of the intestinal and hepatic forms of the disease, although pulmonary, renal, cutaneous and brain infections do occur. The therapeutic agents used against amebiasis are:

(1) Alkaloids
  (a) Emetine hydrochloride
(2) Iodine-containing oxyquinolines
  (a) Diodoquin
  (b) Chiniofon (Anayodin, Yatren)
  (c) Vioform
(3) 4-Aminoquinolines
  (a) Chloroquine (Aralen)
(4) Organometallics
  (a) Carbarsone
  (b) Thiocarbarsone
  (c) Milibis
  (d) Emetine bismuth iodide
(5) Antibiotics
  (a) Terramycin
  (b) Aureomycin
  (c) Fumagillin
(6) Sulfonamides, given in combination with (2) and (4) for their antibacterial action.

The large number of drugs in use at present for the treatment of amebiasis is evidence that individually they are not entirely effective. An excellent discussion of the status of treatment with the standard drugs is presented by Craig (1944) and McHardy and Frye (1954).

TABLE III

COMPLEMENT FIXATION TEST ON PATIENTS WITH VARIOUS DISEASES

NEGATIVE COMPLEMENT FIXATION TEST

HEPATIC DISEASES	CASES	OTHER DISEASES	CASES
PARASITIC DISEASES:		PARASITIC DISEASES:	
MALARIA	5	ASCARIASIS	1
KALA-AZAR	1	TRICHINOSIS	1
ECHINOCOCCUS CYST	1		
OTHER:		OTHER:	
INFECTIOUS HEPATITIS	16	INFECTIOUS MONONUCLEOSIS	3
CIRRHOSIS	5	CARCINOMA OF G.I. TRACT	2
CARCINOMA OF LIVER	5	RICKETTSIALPOX	1
CHOLECYSTITIS	3	SPRUE	1
		SYPHILIS	2

POSITIVE COMPLEMENT FIXATION TEST

HEPATIC DISEASES	CASES	OTHER DISEASES	CASES
GONORRHIS	1	HEMORRHOIDS, PROCTITIS, SIGMOIDITIS	1
MALARIA	1	CHROMOBLASTOMYCOSIS (?)	1

Hussey & Brown

Prevention

The prevention of amebic infections consists of avoiding food and drink which has been contanimated by unclean hands, insects and through the use of human excreta as a vegetable fertilizer. In poorly sanitated areas, surface water may be a source of infection. The infection is frequently observed among many members of a family and it has been the infected food handler in the family who has transmitted the infection. The prophylaxis of amebic infection depends upon the observance of good personal hygiene, the cleansing of the hands before preparing food, before eating and after the use of the toilet. It is obviously impossible to detect and treat every food handler infected with this parasite, and their education in matters of personal hygiene is therefore indicated. Residents in tropical areas where servants' personal hygiene leaves much to be desired should eliminate any amebic infection among this group and also instruct them in the rudiments of cleanliness. At present there is no prophylactic drug upon which one can depend.

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CLINICAL LABORATORY PROCEDURES AT AN ATOMIC ENERGY RESEARCH LABORATORY*

By BOBBY S. JOSLIN**
and
JEAN SPENCER FELTON, M. D.†

Established in 1943 in the sparsely farmed area of East Tennessee as part of the Manhattan District Project, the Oak Ridge National Laboratory has been engaged in basic atomic energy research, radioisotope production, and in the development of numerous processes related to the application of nuclear energy to defense and peacetime usages. Its original wartime population has quadrupled in ensuing years to 3200 scientists, technicians, craftsmen, and managerial, service, and clerical personnel.

The function of medical supervision of these employees has been delegated to the Health Division, and an active portion of the industrial medical program executed by the Division is centered in the Clinical Laboratory. At the beginning of operation, methods had to be introduced that would provide a measure of the effects, if any, of exposure by the worker to ionizing radiation. This is the story of the Clinical Laboratory and its role in health maintenance at a modern multi-activity research institute.

Early Laboratory Days

One of the first of the measurements effected was the complete blood count taken on all employees at intervals varying from three weeks to three months depending upon the amount of radiation to which the employee was exposed. Initially included was a 300-cell differential count on each new employee, and by means of the serial counts, variations from the individual's baseline erythrocyte and leukocyte levels could be determined. A standard urinalysis accompanied each blood count, and serodiagnostic tests for syphilis were conducted on all new workers.

Hematologic studies in vivo and histologic studies on animal autopsy material were made by the Health Division's Clinical Laboratory for the Biology Division, but as the research effort of the Biology Division grew, laboratories were set up within that division to do this work, freeing the Clinical Laboratory of this responsibility.

As the Oak Ridge National Laboratory expanded—taking on more of the aspects of an industrial organization—the Clinical Laboratory broadened its spectrum of procedures to include more services to the employee. Today we have, we believe, an ideal laboratory—good working conditions and the best of equip-

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ment—all of which makes for greater technical efficiency which, in turn, allows the offering of additional clinical services.

Blood cell studies continue to consume the major portion of time, and control blood counts still are taken on all employees exposed to radiation, but not with the initial frequency, since reviews of the findings over a period of years have shown little, if any, abnormality resulting from occupational causes. Conducting a great number of complete blood counts per day is a time-consuming operation; therefore, the Clinical Laboratory staff has attempted constantly to devise faster, but nonetheless accurate, methods for the technical and clerical handling of this volume of work. Much progress has been made, but efforts toward finding a shorter or newer method continue.

Both hemoglobin and erythrocyte levels are determined by photoelectric methods, and leukocyte counts are made by the counting chamber method, and one hundred cell differential counts are conducted. Record keeping has been simplified by the utilization of a punch card system in place of hand entry files, and by the combining of certain laboratory report forms to a single form.

Erythrocyte Counts

Normally, the most time-consuming portion of the complete blood count is the determination of total erythrocytes. In order to shorten this procedure, a Cenco-Sherd-Sanford Photelometer[1] was procured and calibrated so that erythrocyte counts could be conducted according to the technic outlined by Blum[2] (Fig. 1). The calibration curve for each photelometer must be prepared by the laboratory using the instrument. Great care is to be taken in this procedure, since the erythrocyte determinations resulting will be only as accurate as the calibration curve that has been plotted. The effort will be repaid later by greater accuracy in the counts, and in the time saved per count. With normal care, the calibration curve should be accurate within $\pm 300{,}000$ cells as compared to $\pm 800{,}000$[3] cells by the chamber method. Once the calibration curve is determined, the procedure for the erythrocyte count is carried out in this manner:

(1) Make a 1-500 dilution of blood in normal saline, using a .02 cu. mm. Sahli pipet and a 10 cc. volumetric flask.

(2) Mix thoroughly and pour into tubular reading cell.[4]

(3) Read on photelometer scale and interpret reading from calibration charts into number of erythrocytes per cu. mm. of blood.

After the dilutions are made, 20 erythrocyte counts can be read in about 15 minutes. By the counting chamber method, these would take at least two or three times as long. With this new method and the time gained, efforts could be devoted to other clinical laboratory measurements.

Figure 1. Photelometer in use for determination of erythrocyte counts.

Source of Error

Since the photoelectric reading in this method depends upon size, number, and density (hemoglobin concentration) of the erythrocytes, a source of error is found in the variation in size and hemoglobin concentration of these cells. The calibration curve is plotted, using specimens of blood within the average normal range of mean corpuscular volumes (80-94 cubic microns) which had an average hemoglobin content. Deviation from this average size and hemoglobin concentration may lead to significant errors in the red cell count. The size factor can be eliminated by examination of the red cells on a blood smear used for the white cell differential count. The hemoglobin concentration factor need not be considered unless the specimen of blood shows less than 12 grams of hemoglobin, or a color index outside the range of 0.8 to 1.1.

If one finds that the size or concentration factors are not within the normal range, then it is necessary to repeat the count using the hemacytometer. Fortunately, significant error in the photoelectric count is introduced only in cases of marked variations in the above factors, and can be detected readily. Unlike hospital laboratories whose patients would most likely have blood counts with abnormal findings, the clinical laboratory of an industrial

organization deals mainly with normal, healthy, working employees whose blood counts usually come within the normal range—seldom making it necessary to revert to the hemacytometer method. Therefore, we can conclude that the photoelectric method is the fastest and most accurate method for accomplishing hematologic screening of a large number of employees who demonstrate approximately normal red cell counts.

Hemoglobin Determinations

A Dick-Stevens Hemoglobinometer[5] colorimeter is used for determining hemoglobin content. This apparatus reads directly in grams of hemoglobin per 100 cc. of blood and is calibrated by the manufacturer. The technic used is as follows:

(1) A 1-100 dilution of blood in sodium carbonate is prepared, using a special Dick-Stevens pipet.[6]

(2) Some of this solution is blown into a photo-reading cell.

(3) Grams of hemoglobin per 100 cc. of blood is read directly from the hemoglobinometer scale.

The percentage of hemoglobin (if desired—primarily for an easily understood scale by the patient) is calculated from the grams of hemoglobin, using a previously prepared table of values, where 15.8 grams equal 100 per cent for men, and 13.8 grams equal 100 per cent for women. The color index is then determined using the Upjohn® Hematorule (slide rule).

Leukocyte Counts

The white blood cells are counted by the hemacytometer method. The differential count is estimated from a 100 cell count on a Wright's stain blood smear.

Laboratory Numbering of the Blood Counts

In using photoelectric methods, it is more satisfactory to keep together all samples that are to be read on the same photelometer. Using the Cenco-Sheard-Sanford Photelometer for the red cell count, and the Dick-Stevens Hemoglobinometer for the hemoglobin determination, necessarily leaves the leukocyte count to be determined separately, and the differential smear to be stained later and examined.

Breaking the blood count into its constituent parts, keeping each part separate, required a numbering method and holders to keep the parts numerically identifiable so that they could be combined later and reported as the complete count for a given specimen of blood. Racks were designed and numbered for this procedure, so that the number on the hematology report form would correspond to the number on the red cell volumetric flask, and the hemoglobin and white cell pipettes. The blood smears are dated and numbered comparably. When determinations are

to be made, the desired rack of numbered samples is taken to the section of the laboratory set up for a particular test, and confusion in sample identification is avoided.

Collection of Specimens

The greater portion of the blood specimens is collected in the clinical laboratory. A special table was designed for this work which has all the necessary equipment within convenient reach (Fig. 2). Specimens are collected from new employees as part of the preplacement physical examination, from employees terminating their jobs, and from employees reporting for their annual multiphasic screening examinations. For certain employees involved only with the semi-annual routine laboratory procedures (complete blood count and urinalysis), arrangements have been made to collect specimens in the building where the individual works. Containers were designed to carry the equipment needed and the specimens that were collected, and transportation was arranged for the technician and her equipment. In this way, many man hours have been saved by employees no longer required to make the trip to the Health Division building for laboratory service.

Figure 2. Table used in clinical laboratory for collection of blood specimens.

Field Equipment

One container was designed to hold alcohol, diluting fluid for white cell counts and hemoglobin, gauze squares, a removable rack for 10 cc. volumetric flasks (red cell counts) microscope slides, a slotted rack for blood smears, pipets, etc., for collecting a complete blood count (Figure 3).

Figure 3. Portable container and equipment used for the collection and transportation of blood specimens for use in determination of erythrocyte counts in the field.

Other containers were designed to hold filled leukocyte and hemoglobin pipets. These boxes were provided with spring clamp pipet holders to prevent leakage during transportation. Two types of spring holders had to be used in order to accommodate the pipets of different length. The pipet boxes were hinged on one side to form a carrying case (Fig. 4).

Each pipet holder and volumetric flask bears an identification number so that the constituent parts of the blood count can be handled in the same manner as the specimens collected in the

Figure 4. Portable container and equipment used for the collection and transportation of blood specimens for use in the determination of leukocyte counts.

clinical laboratory. A certain series of numbers is assigned to the field counts (such as numbers 1 through 30), and another series to the counts collected in the clinical laboratory (31 through 45), so that at the time the counts are to be made they can be combined to run consecutively 1 through 45.

Boxes were made to hold seven-ounce urine specimen bottles. To avoid embarrassment on the part of sensitive employees, one of these boxes is placed in the men's rest room, and another in the women's rest room, so that the employee need not deliver his specimen to the technician in full view of his fellow workers. Caps, bearing the employee's name and badge number, are placed on the filled bottles to forestall spillage en route to the clinical laboratory.

Participation in the Multiphasic Screening Program

A multiphasic screening program is carried out by the Health Division annually for all employees. The clinical laboratory pro-

cedure in this health maintenance effort include:

1. Complete blood count.
2. Urinalysis.
3. Serodiagnostic test for syphilis.
4. Blood typing (done at time of pre-placement examination only).
5. Rh factor determination (done at time of pre-placement examination only).
6. Electrocardiogram.
7. Ballistocardiogram.

The remainder of the program, performed by other sections of the Division, includes a chest film, test for visual acuity through use of a stereoscopic testing instrument, completion of an interval history form, and an audiogram.

1. Complete Blood Count

The complete blood count is carried out as described previously.

2. Urinalysis

Urine specimens are collected, and are analyzed simultaneously with those collected in the field. The presence of albumin is determined by the heat and acid test, and all positive samples are checked by the Roberts test. Sugar is detected by the Benedict test, and all specimens showing 4+ sugar are checked for acetone and diacetic acid. A centrifuged sediment is examined microscopically and as red cells in urinary sediment often are crenated or disintegrated in specimens a few hours old, a test for occult blood is run on the sediments using the Orthotolidine test.

3. Serodiagnostic Test for Syphilis

Although the clinical laboratory is a State-approved laboratory[9] for the performance of the Kahn test, arrangements have been made with the Tennessee Department of Public Health Laboratory, located in Knoxville, Tennessee, to accomplish these tests on blood specimens submitted to them. This service, offered by the State of Tennessee, is used advantageously, thus enabling the technicians of the Clinical Laboratory to give other services to the employees.

4. Blood Typing, and 5. Rh Factor Determination

In cooperation with the Oak Ridge Chapter of the American Red Cross and the Civil Defense effort, all employees are blood typed and are provided with wallet-size cards stating their blood type and Rh factor. A blood type file is maintained by the Health Division so that donors can be referred promptly to the hospital when there is a need. The Rh factor is determined, including the types Rh, Rh_o' and Rh_o''. These, of course, are carried out only at the time of employment, and are not repeated.

6. Electrocardiogram

An electrocardiogram is made on each employee, using a direct

reading instrument, which obviates the time-consuming film development of the string galvanometer type. Both standard and unipolar limb leads, and unipolar precordial leads in positions 1, 4 and 6 are recorded. These cardiographs are mounted on window slot cards and are kept as a permanent part of each employee's clinical record. The height, weight, age, blood pressure, and occupation are also recorded on the mounting card. Electrocardiograms are scheduled for every 15 minutes, and only one technician is assigned to the electrocardiogram appointments. The employee's blood pressure and other identifying information are recorded by the technician assigned to the drawing of the blood specimens. To help maintain the time schedule, a new suction cup type of electrode[10] is used which saves the time required for the strapping on of the older type of electrode.

7. Ballistocardiogram

A study of ballistocardiography is now in progress, and ballistocardiograms are made on all employees over the age of 40. A Dock Ballistograph is used which records the ballistocardiogram and the spike of the QRS interval of the electrocardiogram simultaneously.

Other Procedures of the Clinical Laboratory

The procedures outlined constitute the major portion of the work, and these have been set up on a routine basis. Other examinations are carried out by special request of a staff physician, and include determinations in bacteriology, blood chemistry, basal metabolism, parasitology, etc.

Special studies undertaken by the Health Division often include procedures to be accomplished by the Clinical Laboratory. These, in time past, have included such tests as the phenolsulfonaphthalein kidney function test, sedimentation rate using different anticoagulants, and also the most recently added test, the tubeless gastric analysis.[11]

An initial study of the food handlers of the Laboratory cafeteria involved stool examinations for parasites and ova, and cultures for the Eberthella, Shigella and Salmonella groups.

Hypertension appearing among a certain group of employees led to special studies including Fishberg concentrations, complete blood counts, urinalyses, phenolsulfanophthalein kidney function studies, urea clearance, urea nitrogen and creatinine determinations.

A blood sugar survey, as part of the annual National Diabetes Detection Week, was completed recently. Blood specimens were collected from each employee and the determinations were carried out by the Folin-Wu method, using the Klett-Summerson photoelectric colorimeter. The 1-10 protein free filtrate was made up in the 15 cc centrifuge tube and separated by centrifugation.

Approximately 100 employees were seen per day, with the determinations run in groups of twenty.

Working Environment

The clinical laboratory is housed in a large room 14 feet by 22 feet. The monochromatic color scheme, in various shades of blue, follows that of the Health Division building, with dark blue woodwork, light blue walls, and white ceilings. The sinks, drain boards, and cabinet work tops are of stainless steel, all of which were designed by the Clinical Laboratory staff with usage factors and storage space in mind, and were constructed by the Engineering and Maintenance Division of the Laboratory. Each technician's desk and work table is covered with light blue Textolite.® The electrocardiogram and basal metabolism rooms adjoin the laboratory proper and are air-conditioned.

The staff of the Clinical Laboratory includes four technicians who are under the supervision of the Medical Director. This averages one technician per 800 employees. Laboratory hours are from 8:00 a.m. to 4:30 p.m., with no night-call duty. The work program is set up on a rotational basis: one technician is assigned to electrocardiograms, ballistocardiograms and basal metabolisms; one to the collection of field specimens and urinalysis; one to the collection of specimens in the clinical laboratory, and the performing of blood counts; and one technician is assigned to special requests and studies (blood chemistry, bacteriology, etc.). The differential blood smears are distributed equally among all four technicians.

Conclusions

The Clinical Laboratory was established as a hematology laboratory to accomplish control blood counts on the employees of the Oak Ridge National Laboratory, because of the ionizing radiation to which they might come in contact. In the ten years that it has existed, newer and faster methods of managing the work have been sought and many more services have been offered to the employees.

As industry becomes more complex and more health-conscious, the Clinical Laboratory becomes more of a necessity in the maintenance of the well-being of the individual industrial worker. At the Oak Ridge National Laboratory, the contribution made by the medical technicians has been reflected in the absence of harmful effects from work contacts in the newest member of the industrial family, atomic energy.

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HINTS From The Affiliated Society Publications

Polyethylene pipet jars resistant to acids, alkalis, and detergents, are obtainable from the Scientific Products Division of the American Hospital Supply Corporation. Their cost is well worth the number of pipet tips they save.

★ ★ ★

Alconox, a detergent sold by Alconox Incorporated of Jersey City, New Jersey, is an efficient cleaning agent for micro slides. Wiping is eliminated. Moss and McQuown in their, "Atlas of Medical Mycology," recommended using Alconox as a mounting medium for fungi because it minimized artifacts due to bubbles.

★ ★ ★

Inexpensive disposable lancets are sold by Meinecke and Company, Incorporated, of New York City.

★ ★ ★

Haemo-Sol in 1 per cent aqueous solution plus 4 per cent phenol is a good disinfectant and cleaner for bacteriologic pipets. This solution can be used successfully for nearly a week. Pipets should be immersed at least twelve hours before rinsing and drying.

★ ★ ★

An interesting rabbit box is marketed by the Louis C. Herring and Company of 1219 Kuhl Avenue, Orlando, Florida. Because of its adjustable nature, the box holds animals of various sizes firmly.

★ ★ ★

If a small bit of white paper marked with dilute (10 percent) hydrochloric acid are placed inside packages or materials to be sterilized by hot air, the marks become brown or black beginning at about 165 degrees Centigrade. If the marking on the paper has not turned brown, the article may be suspected of not having been sterilized.

From the *Journal of the Wisc. Ass'n of M. Technologists*, July-August 1954.

A SIMPLE DEVICE FOR GAS FREE INTRODUCTION OF FLUIDS IN VAN SLYKE BLOOD GAS ANALYSIS

F. RAPPAPORT, Ph.D. and F. EICHHORN
Beilinson Hospital, Petah Tiqua, Israel

There has been felt for a long time, need for a simple method of introducing blood or other biological fluids into the apparatus for gas analysis of the van Slyke type. The previously accepted procedure calls for the use of an Ostwald pipette tipped with a soft rubber ring. This allows direct transfer of the sample into the chamber of the apparatus.[1] In this method the rubber tubing seals the sides of the upper stopcock opening and prevents any of the atmospheric gases from coming into contact with the sample on its way from the pipette into the chamber.

We have recently found that the rubber ring made of rubber tubing may be successfully replaced by a tip made of polyethylene tubing. The use of thin polyethylene tubing instead of heavy rubber prevents unnecessary stirring of the extraction fluid above the stopcock and the stopcock surface is not liable to damage by the glass tip of the pipette.

The protecting tip is prepared as follows: Polyethylene tubing of suitable bore is drawn to a capillary as shown in Fig. 1. The

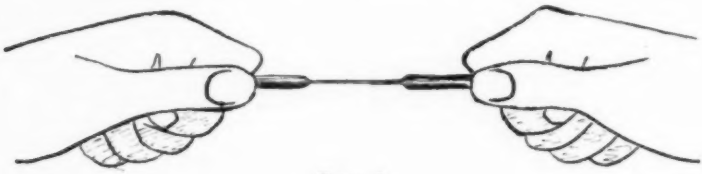


Figure 1.

tubing is cut according to need in the capillary portion, next to the beginning of the constriction. The tip of the pipette is inserted into the wide bore end of the cut piece. In this manner a plastic extension of the pipette tip is achieved (Fig. 2). The plastic capillary end may be easily inserted into the capillary opening of the stopcock. One may also use a polyethylene ring pulled over the tip of the pipette so that the tip itself remains free and the polyethylene sleeve seals the place of contact of the tip of the pipette with the stopcock opening (Figs. 3A, and B).

Polyethylene tubing, constricted by stretching, may also be used for other purposes, such as keeping reagents free from gas[2] or for removing mercury or other fluids from places where the access is by capillary only. In such cases, when the capillary tip

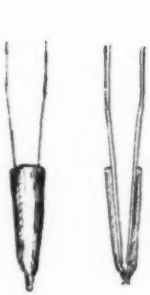
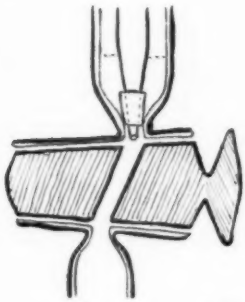


Figure 2



A

Figure 3 B

is made of glass it may often break and the fragments be collected with the fluid. Polyethylene capillary extension, on the other hand, is pliable and unbreakable.

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HINTS From the Affiliated Society Publications

There have been several inquiries about desicoted glassware. Desicote is an organo-silicon compound, originally formulated to give added convenience and high measurement activity. Glassware treated with Desicote requires no cleaning—saves countless hours of washing, rinsing, and drying. There is no drainage time because aqueous solutions cannot adhere to Desicote surfaces. Volumetric glassware treated with Desicote will deliver its entire contents without leaving a film on glass walls.

Desicote is manufactured by Beckman Instrument Co., South Pasadena, California, and may be obtained through most laboratory dealers.

THE GAVEL

In the last two messages for the Gavel I have tried to stimulate interest and thoughtful inspection of our professional responsibilities in the broad aspects of education. Our increasing duties in teaching and student training programs as well as the need for continuous individual growth both in scientific endeavor and in professional attitude were briefly mentioned.

Another facet of this broad subject of education presents the question of how to make this knowledge effective. That effective use of acquired knowledge of techniques for our daily work is made, is immediately apparent and, of course, of prime importance. But beyond this, creating awareness in prospective students and consumers of our services for the need of accurate intelligent laboratory service as an indispensible part of the work of the whole medical team for the prevention and cure of disease is a duty that must not be disregarded by any one of us. This actually requires very little effort other than illustration and example as we make daily contacts with the public in our jobs.

Use of knowledge can be effectively promoted by our professional organization. Once graduated and working, many of us have little opportunity to see our colleagues. This is true not only of those working in isolated communities but also those working in larger cities. A professional organization provides a means to promote unity and to foster good-fellowship. It has been said that an organization is a process by which the individual is helped to get the most out of himself and by which he can do the greatest possible amount of good in the community.

To keep the high standards of our education we must have faith in our future and in our own ability to help the progress of our profession. By individual enthusiasm in making this knowledge effective will good work be stimulated elsewhere. We must not be indifferent or complacent or allow local selfish interests to narrow the influence of our work which exists for the good of the whole community.

Ruth Hovde

IN MEMORIAM

On October 27, 1954, many friends of Sister M. Alcuin (Arena), OSB, were shocked and saddened by the news of her death. It seems incredible that we shall be without the leadership and strength of convictions she has shown in her long affiliation with her state and national societies. Her outstanding accomplishments during this time bear repeating to encourage us in continuing the progress of our profession.

Sister Alcuin's teaching experience was vast, most of it on the college level. She had a total of twenty-four papers published in the *American Journal of Medical Technology*, *Hospital Progress*, *Minnesota Medical Technologist* and *The Techni-caller*. Her three handbooks on clinical laboratory work have been published and revised. A fourth is in manuscript form. She recently completed the manuscript for *Frontiers in Medical Technology* which deals with the progress of the profession during the past twenty-five years.

Sister Alcuin's membership in the American Chemical Society, American Association of University Women, American Society of Medical Technologists, Minnesota Society of Medical Technologists and Alpha Mu Tau Fraternity indicated her unfailing energy in the matters pertaining to Medical Technology. Her interest was manifest in the organization of the Minnesota Society in 1937. She was the first president and served subsequently with almost every committee of MSMT and its district organization, the Arrowhead Society. In 1948 she assisted in the establishment of a Service Fund designated as a loan fund for students in Medical Technology. Her activities were not restricted to Minnesota. She served ASMT as vice president, recording secretary, and as an associate editor of the Journal, as well as on numerous committees, including the Program Committee of the sixteenth annual convention. She was a member of the Program Committee of the coming twenty-third annual convention.

As the Journal goes to press, we are saddened by the news of the death of Miss Mary Frances Gridley on December 31, 1954. Miss Gridley had resigned from the chairmanship of the Research Committee in September because of illness. At the time of her death she was a member of the Editorial Staff of THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY.

Both have received national acclaim for their work and accomplishments. Their memory will be an inspiration to us.

THE WHY AND HOW OF STARTING A SCHOOL OF MEDICAL TECHNOLOGY.

Sister Charles Adele, S.C.N. St. Vincent's Infirmary, Little Rock, Ark. From Hospital Progress, December, 1953.

The EDITOR'S NOTE prefacing this article stresses the point of this undertaking as not so difficult as it is sometimes thought, and hopes that the article will inspire others to consider opening new schools.

The Sister compares the "Land of Plenty" where she had been missioned for fifteen years with "The Land of Opportunity" where she was later sent, and which provided the background for this venture in starting a new school. "The Land of Plenty" had many approved schools and so personnel and professional help was no problem at all, but when she reached "The Land of Opportunity" she was faced with a new problem. There was no approved school and a great lack of trained people. In fact, there were none available. She was confronted not only with the needs of their own hospital, but also of the needs of many doctor's offices and others as well. The only alternative was to establish a school.

Following is her outline of the steps required to organize a school.

1. Getting information.
   a. Contacting schools already existing for course outlines, requirements, etc.
   b. Contacting the Council on Medical Education and Hospitals of the AMA. Through them they received a copy of "The Essentials of an Acceptable School for Medical Technologists." This pamphlet gave them two organizations primarily concerned with the training of Medical Technologists. First, the Council on Medical Education and Hospitals of the A.M.A. which functions by inspecting, reporting and approving schools, while the other, the Board of Registry of Medical Technologists investigates and certifies the competency of the technologists. The pamphlet also gave them standards of organization, requirements as to space, light, and equipment. Much beneficial help was received from "A Curriculum for Schools of Medical Technology," the editors of which are Dr. Israel Davidson and Dr. Kurt Stern. The book is approved by the Board of Registry of A.S.C.P., and may be purchased from them. The book provides an excellent bibliography on the organization of laboratories, also a chapter on examinations of great value to students and teachers.
2. Trial curriculum.

   Each hospital must decide for itself how to divide time between various services so as to give their students adequate preparation and practice. There follows next an actual outline of the curriculum with the hours to be devoted to each subject.
3. Application for approval.

   After meeting the requirements for an approved school, the next step is to make formal application to the Council on Medical Education and Hospitals of the A.M.A., 535 N. Dearborn St., Chicago, 10, Ill. With the great need that exists for approved schools, approval is not as speedy as would be expected. This section gives all the set-backs and delays which may occur before being granted final approval.
4. Recruitment of Students.

   Posters
   Talks in schools
   Publicity in various publications
   Also a word here and a word there brought in some applications.

Up to May 1952, there were 496 approved schools in existance. There were 18,035 medical technologists registered, and there were 36,000 available laboratory positions. So, it can well be seen that the present number of approved schools is inadequate. Recruitment is essential, but of no value if schools are not available to receive students when recruited, so it seems only logical to answer the question "Why Start A School of Medical Technology?" is finding out how to do it. St. Vincent's Hospital did it and expects to train enough technologists to meet their own needs. Many others could do the same.

AMONG THE NEW BOOKS

REVIEW OF MEDICAL MICROBIOLOGY: by Ernest Jawetz, Ph.,D., M.D., Professor of Bacteriology and Lecturer in Medicine and Pediatrics, University of California School of Medicine, San Francisco, Joseph L. Melnick, Ph.D., Professor of Epidemiology, Yale University School of Medicine, Edward A. Adelberg, Ph.D., Assistant Professor of Bacteriology, University of California, Berkeley. 1954. Lange Medical Publications, Los Altos, California. 360 pages. 92 illustrations and photographs. Many charts and tables, $4.50.

The REVIEW is set up with the idea that it will be revised and brought up to date every two years. The first 199 pages are devoted to bacteriology. Besides the usual chapters on cytology and cultivation, there are extensive notes on variations, metabolism and antibacterial agents. Miscellaneous pathogenic microorganisms are discussed, as are spirochaetes, the rickettsia, viruses, and fungi. Generally each organism is named and its morphology and identification given. Pathogenesis and clinical findings are described. Diagnostic laboratory tests are listed. Finally, treatment, epidemiology prophylaxis, and control are added. Some of the newer laboratory techniques are described in detail, especially in the chapters on serological diagnoses. There is also a series of chapters devoted to the various types of disease, giving the vectors, the properties of the causative organisms, including descriptions, reactions of physical agents, animal susceptibility, and antigenic properties.

Although the authors' intention was primarily to direct the information to the medical student and the physician, they have succeeded admirably in making up an excellent summary for the use of the technologist.

THE BIOCHEMISTRY OF SEMEN: by T. Mann, M.D., Sc.D., Ph.D., F.R.S., Reader in Physiology of Animal Reproduction in the University of Cambridge. 1954. London: Methuen & Co., Ltd., New York: John Wiley & Sons, Inc. 240 pages. 7 plates. 16 text figures. $2.90.

This book is too highly specialized to be of much value in the routine laboratory. However, it will give the clinician worthwhile information as he investigates the properties relating to the chemical mechanisms underlying the viability and senescence of living animal cells. Chapters of greatest practical worth are those on the Two Components of Semen, and the Chemical and Physical Properties of Semen.

PRACTICAL CLINICAL BIOCHEMISTRY: by Harold Varley, M.Sc., F.R.I.C., Biochemist, Manchester Royal Infirmary, Lecturer in Clinical Pathology, Manchester University. 1954. New York. Interscience Publishers, Inc. London. William Heinemann—Medical Books, Ltd. 551 pages. 70 illustrations and charts. 31 tables. Appendices. $6.50.

With an introductory chapter on the collection of specimens and "Some General Techniques," this volume has a good beginning. Many points are brought out that otherwise seem to rate only "word of mouth" instructions. The relationship of chemical testing of urine and blood is well established and simply described. Very "functional" will be as good a description as any for a book that any laboratory library will find well worth the purchase price. Although the same techniques will be available elsewhere, they are so placed here that where time is considered, the saving will be considerable.

BENJAMIN FRANKLIN'S "SOME ACCOUNT OF THE PENNSYLVANIA HOSPITAL": Printed in Facsimile, with an Introduction by I. Bernard Cohen. 1954. Baltimore. The Johns Hopkins Press. 40 pages. $3.25.

You will want this for your personal library if you like to apply past history to our own time. The Account is reproduced in facsimile from a rare original. It provides an excellent example of the type of printing done by Benjamin Franklin. The Account was first presented by Franklin as a fund-raising project, and I. Bernard Cohen says in his introduction: "The

arguments advanced by Franklin in support of the hospital are compelling, and it is difficult to believe they were conceived two hundred years ago and not yesterday." Franklin's thesis is that "an organized group of men working for a common goal can do more good in the world than if each man worked to help his neighbors by himself."

COTTON MATHER, FIRST SIGNIFICANT FIGURE IN AMERICAN MEDICINE: by Otho T. Beall, Jr. and Richard H. Shyrock. 1954. The Johns Hopkins Press, Baltimore. 241 pages. Illustrated. $4.00.

According to the preface, the aim of this book is not only to present Cotton Mather as "the first significant figure in American medicine," but to "provide a detailed case study of medical thought as can be observed in the English Colonies at the beginning of the eighteenth century." Once we read through the first sixty-six pages of explanatory matter and get into the chapter which treats of the nature of "THE ANGEL OF BETHESDA," followed by that on the "Advent of Preventive Medicine," and finally read the "Proposals for printing a book entitled: "THE ANGEL OF BETHESDA," a 103 page Appendix, we find indeed a most fascinating "Essay upon the Common Maladies of Mankind." Even more absorbing, is "a rich Collection of plain but potent and approved Remedies for the Maladies." Although this is good reading, Cotton Mather's religious attitudes frequently manifest themselves. We wondered if the medical laboratory had any place in this treatise. We could not find any reference that might cause us to suspect even the earliest dark corner of such an aide to the physician of the day.

ORGANIZATION AND ADMINISTRATION OF BLOOD BANKS: by 31 leaders in the field of Blood Banking, members of the American Association of Blood Banks. 1954. The American Association of Blood Banks, 3707 Gaston Avenue, Dallas, Texas. 114 pages. $4.00.

This book can best be reviewed by quoting from the preface: "It is not the desire here to appraise the merits and defects of the different kinds or systems of blood banks, rather to described them as they are. Nor is it the intent or purpose to state what type of blood is needed nor to specify how it should be organized for any particular hospital or community." The experiences presented here are by the pioneers in blood banking: John Elliott, Sc.D., Julius Davenport, Jr., M.D., Bernice M. Hemphill, G. Albin Matson, Ph.D., Joseph M. Potts, Marjorie Saunders, L.L.B., and all those others whose contributions give substance to this comparatively new field. This should be in every hospital and blood banking institution in this country.

THE PHYSICIAN AND HIS PRACTICE: edited by Joseph Garland, M.D., Editor THE NEW ENGLAND JOURNAL OF MEDICINE, containing articles by 19 authors. 1954. Little, Brown and Company. Boston. Toronto. 270 pages. Tables. $5.00.

Besides such phases of the physician's practice as "Choice of Opportunities," "General Medicine," "Group Practice," "Family and Community Relations," as well as "Drugs and Medical Supplies," "The Office and Related Business Aspects," and "Auxiliary Services," we find a chapter devoted to "Laboratory Facilities." This describes the various aspects of a small laboratory, established for the primary purpose of office routine as contrasted with hospital facilities.

ALSO RECEIVED: CLINICAL ASPECTS OF THE AUTONOMIC NERVOUS SYSTEM: by L. A. Gilfillan, Ph.D., M.D., Associate Professor of Anatomy, Graduate School of Medicine, University of Pennsylvania. 1954 316 pages. 42 illustrations. Little, Brown & Company, Boston. $6.50.

ABSTRACTS

CROSS-MATCHING OF BLOOD IN THE PRESENCE OF DEXTRAN

Nancy A. Marston. (Central-Middlesex Hospital, London). Lancet II, 688 (1954)

The interference of dextran in cross-matching procedures depends on the concentration of dextran in the blood serum, on its molecular-weight distribution, on the patient into whom the dextran is administered, and on the cells which are to be transfused. The conclusion is drawn that small quantities of dextran should not be an interference.

"Intradex," Glaxo, was the dextran used in this hospital as the plasma-volume expander. Normally samples of blood are obtained before administration of dextran. Occasionally this was forgotten and it was thought desirable to find out how often a sample of blood caused rouleau formation sufficient to interfere seriously with cross-matching after the infusion of one or more bottles of dextran.

Fourteen patients (13 were Rh_o positive-Group O), were given ½ to 2 bottles of dextran and when the infusion had been completed a sample of blood was taken. The serum was tested with Group O, Rh_o positive cells by the slide method and by tube methods using cells suspended in saline solution and albumin. Rouleau formation was not observed in eight cases who had received ½ to 2 bottles of dextran: minor roleau in 3 cases who had received 1, 1 or 2 bottles of dextran; and marked rouleau in three cases who had received one bottle each of dextran.

The rouleau formation was quite distinct from agglutination and no more than that observed in patients who have not received dextran. The presence of dextran increased the agglutinating action of antibodies shown with an anti-A serum with a titer of 256 in saline solution which showed definite agglutination to a titer of 4096 when titrated in a medium containing 3% dextran.

For most patients it seems reasonable to cross-match blood in the usual way. When 1000-1500 ml. of dextran has been given and difficulty is encountered the Sheffield technique, shown by Dodge (1952) to be the most reliable, can be used:

In a Kahn tube place equal volumes of:

Recipient's serum	2 - 5 drops
Donor's cells, 5% suspension in saline solution	2 - 5 drops

Mix and incubate at 37° C for 1-1½ hours.

Remove supernatant fluid as completely as possible and add an equal volume of

20% bovine albumin	2 - 5 drops

Mix and incubate for ½ to 1 hour.

Examine microscopically in the usual way.

CROSS-MATCHING OF BLOOD IN THE PRESENCE OF DEXTRAN

John Wallace. (Giffnoch, Renfrewshire). Lancet II, 761 (1954).

Difficulty to differentiate from true agglutination the intense rouleau which occasionally developed with cross-matching procedures with the blood from recipients of dextran. Of 7000 patients cross-matched blood had to be tested immediately after the infusion of dextran from 31 cases. In 5 of these intense rouleau formation took place when the recipient's serum was incubated in tubes at 37° C with homologous A-B-O and Rh donor red cells suspended in saline and in 20% bovine albumin. The rouleau formation could not be broken up but the indirect anti-globulin test was negative.

The prior administration of dextran may cause difficulty in determining the recipient's blood group. The rapid emergency slide methods of Rh (D) grouping which calls for heavy cell suspensions in serum may give dangerous false positive results. Apparent discrepancies in A-B-O group may arise from marked rouleau formation when the recipient's serum is tested with standard A and B cells.

These difficulties, however infrequent, stress the necessity of having these tests performed only by a thoroughly trained and experienced staff and the importance of obtaining a sample of the recipient's blood prior to the administration of dextran.

EFFECT OF HIGH FAT FEEDINGS ON VISCOSITY OF THE BLOOD

Roy L. Swank. (Univ. of Oregon Med. School, Portland). Science 120, 427-8 (1954)

Hamsters were used in these observations. The *in vivo* changes were observed in the cheek pouch anesthetized with urethane and recorded in colored motion pictures. The purpose of this report was to show that significant increases in the viscosity of blood from the vena cava accompanies the aggregation and adhesiveness of red blood cells observed in the cheek pouch after fat meals.

The maximum adhesiveness and aggregation of the red blood cells occurred two to four hours after the peak of the plasma lipemia and lasted for several hours after the lipemia cleared. The author states that it would appear that the maximum increase in the viscosity also occurs after the peak of the lipemia.

The possibility is brought out that these findings may have some effect as a factor in human disease, particularly in chronic vascular and thrombotic diseases and in multiple sclerosis.

A DEVICE FOR DETERMINING TIME AND TEMPERATURE OF STERILIZATION IN THE AUTOCLAVE OR HOT-AIR OVEN.

John H. Brewer and C. Baxter McLaughlin. (Biological Research Laboratory, Hynson. Westcott & Dunning, Baltimore, Md.). Science 120, 501-2 (1954).

Specially constructed hour-glass type tube with a side-arm by-pass tube were

evaluated for use as a check on proper sterilization in the autoclave. Repeated tests showed that when these tubes contained one gram of succinic anhydride the chemical would melt and flow at 120° to 121° C. in 15 to 17 minutes. From these observations the authors believe that they have an apparatus that will truly indicate the predetermined temperature was reached and held for a minimum sterilizing time. The tubes are inexpensive and can be employed in large numbers and may be used by the small laboratory that cannot afford expensive thermocouples.

By constructing a larger tube and placing a higher melting point solid that melts at 166° to 167° C in it, a device for use in the hot air oven may easily be constructed.

A SEROLOGIC AND GENETIC ANALYSIS OF AN r′ r′ (dCe/dCe) PATIENT PRODUCING ANTI-D AND ANTI-c.

Philip Levine, Mary B. Cooper and Elizabeth A. Koch. (Ortho Research Foundation, Raritan, N.J.—General Hospital of Syracuse, N.Y.). Blood IX. 817-23 (1954).

Antibodies of the anti-D and anti-c varieties produced by a transfusion and pregnancy, respectively, were identified by cross absorption experiments. The serum of the patient was diluted 1:2 and absorbed once with equal volumes of washed packed red cells sediment for one hour at 37° C. The supernatants and the unabsorbed serum were titrated and tested by the antiglobulin technic of Coombs. The specificities of the two supernatants were confirmed in tests with numerous bloods of varying Rh-Hr antigens.

The immunized mother (propositus), whose husband was of genotype dce/dce, possessed the very rare genotype dCe/dCe, each of her parents, who were related to each other as fourth cousins, contributing the rare chromosome dCe.

Attention is brought to the frequency this difficulty may have. For in a caucasoid population of 150,000,000 one may expect 15,000 individuals of the rare genotype dCe/dCe and somewhat more than 7500 exposed to the antigenic stimuli of pregnancies and/or transfusions with potential capacity to produce the same combinations of antibodies, i.e., anti-D and anti-c.

It is suggested that the larger blood banks might identify such individuals (at least groups O and A) who can serve as donors. For the protection of patients genetically endowed with rare types of blood it is important to select from the Rh-negative list the rare homozygotes for C and or E; i.e., genotypes dCe/dCe or dcE/dcE. The screening of donors with anti-D only is advocated. And then further tests carried out with anti-C and anti-E for the detection of subtypes dCe (rh′) and dcE (rh″′). Further tests should be made to determine the genotype.

It is suggested that if this procedure is adopted, it will be possible to have a list of such individuals (at least O and A) who can serve as donors for selected cases. These may be listed with a central clearing bureau organized first at a national level and later extended, perhaps through the facilities of the World Health Organization. Unless this is done failure will result in obtaining compatible donors even when the antibodies are identified.

PHASE MICROSCOPY 1950-1954

Oscar W. Richards. (American Optical Co., Research Dept., Southbridge, Mass.). Science 120, 631-9 (1954)

An analytic summary of some 200 publications showing the usefulness of phase microscopy in the study of colorless transparent or nearly colorless transparent materials containing detail composed of small differences in optical path (refractive index × thickness).

Three illustrations are given to demonstrate the principles. Conversion of a bright-field microscope into a phase microscope may be done by replacing the condenser with a phase condenser containing an annulus and the objective with a phase objective containing a diffraction plate. No change is required in the rest of the equipment. The preparation of the diffraction plate is outlined. Details of construction, theory and application of variable and color contrast instead of fixed diffraction plates are obtainable in the references outlined in this review. The phase microscope with the electron microscope give considerable information. Basic orientation is obtained with the phase microscope and considerable detail with the electron microscope. Motion pictures of tissue cultures with phase microscopy have postulated *in vivo* knowledge.

Microorganisms especially acid-fast bacteria have been observed with phase microscopy. This method was said to be twice as good as bright-field for finding tuberculosis bacteria in some 2028 specimens.

Numerous cells and tissues have been examined by various investigators with the phase microscope. Their findings reveal many interesting reports.

The medical profession is using the phase microscope in the clinical analysis of urine sediments, vaginal exudates, feces, semen, sputum, cerebrospinal fluid, blood cells, and red, white, reticulocyte and platelet cell counts; protozoology for cysts and active animals; bacteriology for unstained, living organisms, agglutination, virus and inclusion bodies; helminthology, identification of eggs and diagnostic details of adults; biopsy specimens, especially bone marrow and spleen; identification of fungi and fungus infections; normal and pathological histology, tumor diagnosis, phagocytosis, transplantation and regeneration; and measurement of unstained, living micro-organisms.

Phase motion pictures of tumor tissue showed living leucocytes within the tumor cells. Seyforth suggests that the small granules in tumor cells are endospores, from

which microorganisms develop. Martinez concludes that the phase microscope gives the same diagnosis as H and E staining in a study of eight gliomas but much more quickly. Staining methods also were reported cumbersome, damaging to tissue and time consuming.

Attention was also called to the many industrial uses for the phase microscope.

FRACTIONATION OF HUMAN SERUM ALBUMIN USING CONTINUOUS FILTER-PAPER ELECTROPHORESIS

Daniel L. Larson and Robert Feinberg. (Army Medical Service Graduate School, Wash., D. C.). Science 120, 426-7 (1954).

The gel-diffusion technique was used in testing the immunological homogeneity of a protein after continuous filter-paper electrophoresis. An antiserum was prepared against the material under investigation and the antigen and antibody were permitted to react in a gel medium under certain standard conditions. When tested at least three and in some cases six components were found.

The authors state that this type of electrophoresis is a gentle treatment, that it can be carried out in the cold, and it avoids extreme changes in pH and electrolyte concentration. The fractions so obtained can be quantitatively recovered. Also the fractions are physically separated throughout the remainder of the run after the initial contact of the proteins on the paper. This reduces the interaction between dissimilar proteins.

PRELIMINARY OBSERVATIONS ON DIFFERENCE IN CARBOHYDRATE BINDING BETWEEN ABNORMAL SERUM AND URINE PROTEINS OF MULTIPLE MYELOMA.

Elliott F. Osserman and Dolores P. Lawlor. (Columbia Univ., New York). Science 120, 715-6 (1954)

Nineteen documented cases of multiple myeloma were studied using the modified filter paper electrophoresis technique described by Osserman and Lawlor. Eleven showed the characteristic homomolecular myeloma globulin in both serum and urine; six showed only serum abnormality; two showed only urine abnormality. Veronal buffer, pH 8.6, was used. Urine samples were concentrated by dialysis against a 25% solution of polyvinyl-pyrallidone. Triplicate serum and urine samples were run on a single paper, one set (serum plus urine) was stained for protein with bromphenol blue, and the other two sets were stained for lipids and carbohydrates.

Sudan IV and oil red O stains were used for lipids but neither serum nor the urinary abnormal globulins could be shown to contain detectable amounts of fat. Schiff's periodic-acid (HIO_4-Schiff) staining technique was used to demonstrate "protein-bound carbohydrates."

The abnormal serum proteins stained intensely with HIO_4-Schiff, whereas the abnormal urine proteins were HIO_4-Schiff negative. The normal serum pattern shows HIO_4-Schiff positive constituents in the alpha- and beta- globulin region, indicating the presence of the normal muco- and glycoproteins in these areas.

The nonspecificity of the HIO_4-Schiff reaction limits the conclusions that may be drawn from the observations. These results still do not provide an answer to the question of whether the urine and serum proteins are independently elaborated, or whether the urine constituent represents a fragment of the parent serum molecule. The results do, however, point to further investigation.

Paper Electrophoresis of Abnormal Hemoglobins and Its Clinical Applications—A Simple semiquantitative Method for the Study of Hereditary Hemoglobinopathies

Arno G. Matulsky, Milton H. Paul and E. L. Durrum. (Walter Reed Army Medical Center, Washington, D.C.). Blood IX, 897-910 (1954).

It is likely that paper electrophoretic technic may replace Tiselius electrophoresis in most studies of hemoglobin abnormalities. It is a simple and convenient technic for the study of hereditary hemoglobinopathies. In the method described a semiquantitative paper electrophoretic technic is described which allows rather accurate quantitation of the various hemoglobin components by inspection alone. Its clinical application with the more common abnormalities (such as sickle cell trait, sickle cell anemia, C trait, sickle cell-hemoglobin C disease) and a Negro patient with sickle cell-thalasemia disease were discovered. In all other hereditary and acquired anemias studied normal adult hemoglobin (hemoglobin A) was found. In cases of hereditary nonspherocytic hemolytic disease and aregenerative anemia slightly increased amounts of fetal hemoglobin were detected.

Another value of the method cited was its use in studying the red cell life span of transfused cells. Identical apparatus and buffer may also be used for serum protein electrophoresis. It is claimed that the combination of the technics of paper electrophoresis and alkali denaturation offer an adequate, simple and practical tool for diagnosis and investigation of hereditary hemoglobinopathies.

FROM OUR READERS

From G. G. P., Willmar, Minnesota: AN APPARENT CAUSE OF HEMOLYZED SERUM: Recently it seemed well to institute a routine preliminary icterus index determination on all persons being started on Chlorpromazine (Thorazine) medication.

There was no reason to consider that these patients had any form of liver disease; nevertheless, the indices were ranging from 7 to 12 on approximately 50 samples of blood. It soon was apparent that this was due to traces of hemolysis and the usual measures to prevent it were carefully checked. Glassware and needles were dry, glassware chemically cleaned, blood carefully drawn, bubbling avoided, clot firm before centrifuging.

As a last resort, the use of wooden applicators to loosen the clot was abandoned, and clean stiff wires similar to bacteriological needles were used. Indices promptly fell into normal range, and on those samples done in duplicate to compare the two methods it was apparent by gross visual comparison that there was hemolysis in those specimens that had been loosened with wooden applicators.

The only explanation offered is that possibly traces of bleach or other chemicals in the wood caused the hemolysis.

From R. F. (Student) Technologist, Chattahoochie, Florida: DO YOUR N.P.N.'s "BUMP"? If so, try substituting very small pieces of broken china plate for the usual glass beads.

From P. S., New York, N. Y. comes an inquiry regarding "how often and how routinely the bacteriological antibiotic-chemotherapeutic sensitivity tests by the use of sensitivity discs is employed at hospitals and outside laboratories. Send us a card with the name and address of your laboratory (hospital) if you employ this method.*

From M. J. R., Detroit Michigan: To remove stains in cotton uniforms caused by developing solutions used for X-rays and electrocardiographs saturate the stains with a 7 per cent tincture of iodine solution. Then soak the iodine stains out with 25 per cent ammonia hydroxide.

From P. G. McG., Ederton, Wisconsin. FILING PATIENTS' REPORTS: In order to eliminate fumbling through a mass of papers to find all reports on one patient, use individual envelopes. There is now available commercially a 3" x 5" file card envelope but since our report forms are also 3" x 5", we feel that they are not adequate for a hospital laboratory where patients are re-admitted and build up a considerable file. We use a 3⅝" x 6" coin envelope with the flap cut off. This seems best and cheapest for the purpose. The envelopes are filed in alphabetical order and are instantly available to the clinician who desires the old reports on his patient. We feel that this system results in a saving of time and better laboratory service.

MODIFICATION OF THE GONADOAROPIC HORMONE TEST (FROG): In our laboratory we have added one procedure to the standard test for the gonadotropic hormone using the frog. After the test has run prescribed two hours, we collect all the urine in a small beaker or a medicine glass and then centrifuge for two minutes at three thousand R.P.M. We then pour off the supernatant, spread the sediment on a slide and examine for the presence of sperm. We feel that in this way we examine all the urine from the particular frog and that there is very little chance of missing a weak positive.

PERPETUAL INVENTORY: As technologist director of a small laboratory, it is a recurring nightmare that I shall some day find a reagent bottle empty. Often the realization comes when the reagent has not been included on the last order to the supplier.

* To Editor, American Journal of Medical Technology
25 Hermann Professional Bldg.
Houston 25, Texas

To eliminate this situation, we instituted the perpetual inventory system. This is almost ideally suited to the needs of the small laboratory as well as to the large one.

For this procedure we use a 4" by 6" card with columns for "ON HAND," "ORDERED," "RECEIVED," and "USED." Entries are made in the proper column and the corrected balances are entered in "ON HAND." When articles are ordered, an appropriate entry is made and the cards are pulled and placed in the front of the file. When the articles are received, another entry is made and the cards are re-filed in their proper place. We record the name of the article and the catalogue number at the top of the cards and file them in alphabetical order.

Setting up this system requires very little time if done when the lab. is set up initially. If it is established after the lab. has been operating for a time, it requires many hours of off-duty time. I wish to emphasize that this is by no means wasted. There is an old saying that "to make money you must spend money." In this case we may say that by spending this time we may save time. After the inventory is completed it requires only seconds per article to keep it up to date. This time necessary to fill out order pads or other memoranda or to check stock is saved and needed articles are always on hand in the proper amount.

In addition, the technologist is at all times aware of the exact status of his supplies and equipment and, if he desires, the cost of operating the laboratory for any given period.

HERE'S A GOOD CHECK-LIST TO STUDY FOR LABORATORY-PHYSICIAN RELATIONS

From the Journal of the Wisconsin Association of Medical Technologists, January-February 1954.

By WALTER D. HINES

No matter how you look at it, the laboratory technologist is a valuable asset to the physician, whether in a hospital, clinic, or an industrial dispensary. By the same yardstick of measurement, the physician is equally important to the technologist. With the old year having entered the annals of history, and on the break of a new year, it might be wise, then, to study the following check-list for good technologist-physician relations. Allow yourself five points for each question answered in the affirmative. If your total score is less than one hundred, remember that you have 270 days in which to take corrective action.

___Do you consider that the most valuable personal commodity of the physician is his time, and try to conserve this commodity to the best of your ability?

___Do you attempt to make your relations with the physician as pleasant and enjoyable as possible, regardless of your personal opinion of his practices, ethics, or attitude?

___Are your reports to the physician as clear, concise, and complete as possible? Do you strive to improve your grammar? Your rhetoric?

___Do you offer constructive suggestions to the physician when you feel that supplemental information would be desired—and appreciated?

___Do you seek out the advice of physicians, as well as your fellow technologists? Do you consult available text books to obtain a well-rounded knowledge of unusual (and commonplace) subjects?

___Do you attempt to understand the physician's point of view toward laboratory examinations, patients, and other physicians?

___Are your reports of examination checked for errors and misleading information before they are submitted to the physician?
___Are you prompt at supplying information to the physician when he requests it?
___Do you give the physician the attitude that you are taking a "personal" interest in his patients, work, and requests?
___Do you keep the physician informed of new techniques and procedures?
___Do you present a business-like attitude when discussing problems with the physician, especially in the presence of a patient?
___Is your appearance always commendable to your profession___and the physician?
___Are you critical of your fellow-technologists in the presence of a physician, or physicians?
___Do you discuss the practices and ethics of one physician in the presence of another?
___Do you make commitments without proper authority or adequate knowledge of the subject in question?
___Do you attempt to cultivate good physician relations every day, regardless of your personal feelings toward the physician?
___Do you attempt to harmonize the relations between the physician and his patients? Between the physician and the laboratory staff? Between the physician and business management of your institution?
___Are you appreciative of advice and assistance offered by the physician—and do you make him *feel* your attitude?
___Do you attempt to make new policies in order to please, or displease, a particular physician? Do you cater to the whims and fancies of one physician and not to another?
___Are you constantly striving to better your profession, your techniques, and your attitude toward others?

Now add up your score . . . five points for each affirmative answer. If you have a score of fifty, you are lower than average. If you score between fifty and seventy-five, you have an entire year ahead of you for improvement. If you score eighty-five or more, you had better re-check your answers.

MYCOLOGY TEACHING MATERIAL

ADABELLE N. THOMSON, M. T. (ASCP)

Medical Technician, Bacteriology Branch, Sixth Army Medical Laboratory
Fort Baker, California

Material for demonstration in mycology can become a time consuming burden in a busy laboratory unless some means is devised to prepare specimens that can be used over a period of time. The use of formaldehyde killed cultures, PVA mounts of material from cultures and viable stock cultures under mineral oil make it possible to have a wide selection of specimens available for immediate use.

Formaldehyde Killed Cultures. The cultural characteristics of fungus cultures are of primary importance for identification. Pictures are inadequate and living cultures lose their identifying characteristics with age. The possibility of contamination and

infection also limit the use of viable cultures. In order to establish a permanent collection of fungi to demonstrate cultural characteristics the following technic has been devised. A pure culture of the fungus in a rapidly growing phase is inoculated into an oval prescription bottle of two ounce capacity and fitted with a plastic screw cap. The bottle contains a dry slant of Sabouraud's dextrose agar or medium of choice. One loopful of the inoculum is placed on the middle of the slant to obtain a single giant colony. The culture is allowed to incubate until the culture has developed typical growth characteristics. An absorbent cotton plug shaped to fit snugly into the neck of the prescription bottle is moistened with 40% formaldehyde and inserted into the mouth of the bottle, the plastic cap is screwed tightly in place and the bottle sealed. The seal must be airtight in order to retain the formaldehyde gas and to prevent evaporation and drying of the medium. Pale colors, especially pink and violet hues fade in a few weeks but no other change has been noticed in cultures prepared 3 years ago. Petri plates may be used but bottles store to a better advantage and are more easily sealed.

Slides for Microscopic Study. Growth taken from a culture producing typical identifying structures is suspended in a modified Polyvinyl Alcohol (1) mounting medium, placed on microscopic slides and covered with #1 cover glasses being careful to avoid air bubbles. A set of 5″ x 8″ cards containing a brief description of each specimen accompanies the slides.

Viable Cultures under Mineral Oil. The collection of viable fungus cultures can be maintained with a minimum of transferring and subsequent loss of morphology by sealing the cultures with sterile mineral oil (2). Sabouraud's conservation agar deep sticks or shallow slants are inoculated and allowed to incubate until active growth is observed. Sterile mineral oil is added to a depth of 1 cm, or if slants are used, to cover the tip of the agar slant. Most fungi can be expected to be viable after six months storage at room temperature and many of the contaminants have been found to be viable after two years.

The mineral oil is sterilized in the hot air oven at 160° C for two hours (3).

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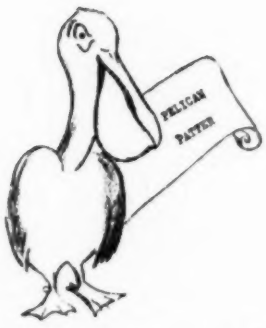

Well, me, I am excited, yes, about how close it is coming—this convention. We have been telling you about all what you going to see, but this time, I want to tell you about something special.

You hear so much about the French Quarter the "Vieux Carré—now I want to tell all those nice technical people who work so hard to make everybody healthy and strong. Now I want to tell them exacty what is the French Quarter. But me—I got to stop and think myself—because the Vieux Carré—she is a different thing to each different people.

We will take first the history. There you find that paradox—the old Nouvelle Orléans. You go on a guided tour or you wander with friends, a guide book in your hand—and right away—you are back in the eighteenth century—with your roots and memories in France—but proud of your new city and anxious to help it grow. You will go in all the famous courtyards and old mansions and visit the Place d'Armes (Jackson Square) with its old cathedral and on each side the Cabildo and the Presbytère—old Spanish government buildings and now a wonderful museum of a by-gone day. Just this month, they opened the "Steamboat Room" with wonderful memories of the old Mississippi river steamboat. You will see places where they auctioned off slaves and where Lafitte had his blacksmith shop.

Then there is second the architecture. People come from all over the world to study and to admire the fine old buildings with their simple, elegant façades and their balconies trimmed with iron lace work hand wrought by slaves.

Then there is, third, Bourbon Street—la rue Bourbon—she is, how shall I say—a night street—and, like the young folks say—a "crazy mixed up" street. There you find some wonderful, gay, authentic jazz bands and old time rag time—lots of fun—and there you find too—sloppy old joints you want to step wide around—but there you find also some fine piano players where you sit and relax and sing about your home town and have a fine time—it's all mixed up—all full of excitement and all gay.

And then—number four—there is—hold on to your pocketbooks—la rue Royale—and the antique shops. There you find everything old—Waterford Crystal Chandeliers—Magnifique!—fine old English furniture—Sèvres figurines—priceless—old silver and china—and jewelry—oh, là—là—the bracelets and dinner rings and necklaces!—and best of all, you don't have to buy—you can just go and look!

The last—there is now what shall I say—the couleur locale—the local color like nowhere else—you walk about and what you see—creole mammies selling pralines, artists everywhere sketching and some waiting to do your portrait—very cheap—very quick—the old French Market—narrow streets and banquettes—not sidewalks—shuttered houses everywhere—convents, studios, courtyards—it is all very wonderful—and this is the part every person finds for himself—ah—almost I wish I were not me, so I, like your friends, could see Nouvelle Orléans for the first time.

Well, next time, I write you again for the last time and then we talk about real plans to make you a happy time here—you know what they call us—the "City That Care Forgot." You going to see!

Au Revoir,
Patate le Pelican.

A.S.M.T. ANNUAL CONVENTION

Louisiana—June 12-17, 1955

Headquarters—Jung Hotel, New Orleans, La.

We, members of LSMT, extend a cordial invitation to each of you to attend the 23rd annual convention in "America's most interesting city," New Orleans.

OUR GOAL—1000 ATTENDANCE—PLAN NOW TO ATTEND!

If you have followed "Pelican Patter" in the past issues of your Journal, you already know about the fun and extra curricular activities that "Patate le Pelican" has planned for you and we hope that your visit to New Orleans will be completely enjoyable. But—lest you think it all play and no work done, let me set down now some important committees and the outstanding features of the very fine program they have prepared for you.

Lucille Godelfer
General Chairman
2928 Bell Street
New Orleans, 19, La.

THE HEART OF OLD NEW ORLEANS
Place d'Armes (Jackson Square)

Left to right, Cabildo, St. Louis Cathedral, Presbytere, Pontalba Apartment (oldest apartment houses in the U.S.) These buildings now house the State Museum.

Program Committee Patter:

I. GUEST SPEAKERS: Some of the outstanding speakers on the program and the subjects they will discuss, follow:

1. Dr. John R. Schenken, Director of Laboratories, Nebraska Methodist Hospital in Omaha, Hematological diagnosis and pathology of leukemia in children.
2. Dr. Stanley Durlacher, Associate Professor of Pathology, L.S.U. School of Medicine, Toxicology.
3. Dr. Rolla Walcott, Medical Director of U.S.P.H.S. Hospital, Carville, Louisiana. Some aspects of Hansen's Disease.
4. Dr. R. J. Muelling Jr., Associate Pathologist, Department of Pathology, Charity Hospital, New Orleans, Electrophoresis.

II. PARASITOLOGY SYMPOSIUM: This will be conducted by Dr. Marion W. Hood, Microbiologist, Department of Pathology, Charity Hospital, New Orleans; Dr. Paul C. Beaver, Associate Professor of Parasitology, Department of Tropical Medicine, Tulane University School of Medicine; and Dr. J. Clyde Schwartzwelder, Professor of Parasitology, Department of Microbiology, L.S.U. School of Medicine.

III. PARASITOLOGY REFRESHER COURSE: A parasitology refresher course will be held on Friday, June 17 at L.S.U. School of Medicine (one block from convention headquarters).
Due to preparation involved, this course will be limited to sixty (60) students. Sufficient material will be available for students to make individual mounts and to actually study the parasites. Reservations for this will be explained in the next issue.
Registration fee for course will be $5.00.

IV. INSTRUMENTATION CLINICS: Dr. R. J. Muelling, Jr., Associate Pathologist, Department of Pathology, Charity Hospital, New Orleans, will conduct instrumentation clinics on:
1. Calibration of pipettes, tubes, etc.
2. Spectrophotometry.
3. Flame photometry.
4. Ph and Potentiometry with titration.

These clinics will be limited to 30 and repeated as many times as necessary so that each one will be afforded an opportunity to actually carry out a calibration procedure.

V. CONFERENCES: These will replace the workshops of former years, and as requested they will not be held concurrently. Plans are being formulated to present the following conferences:
1. Legislation.
2. Education.
3. Recruitment, Public Relations and Membership.

Dr. Ralph Hartwell, Pathologist, Hotel Dieu, New Orleans, member of the Board of Approved Schools of ASCP will participate in the education conference.
Dr. Frank Coleman, Pathologist, Mercy Hospital, Des Moines, Iowa, will participate in the legislative conference.

VI. PROGRAM RULES:
1. **March 15, 1955** is the deadline for the Program Committee Chairman to receive papers from Medical Technologists desiring to present a paper on the Convention Program and to compete for a Convention Award.
2. Only ASMT members are eligible for the ASMT Awards, Parasitology and Hillkowitz Awards. All competitive papers must be presented in person or by proxy at the Convention.
3. All papers submitted to the society become ASMT property. They may be published in the American Journal of Medical Technology. Prize papers from state contests are eligible for awards if they have not been published.
4. Thirty minutes is the time allowed for each paper. Of this time, twenty minutes will be allowed for the reading of the paper and the remaning ten minutes will be for slide showing and discussion. Papers may be abstracted for the program and longer for publication.
5. Indicate on the fly sheet of the paper the following: The awards for which the paper is submitted; author's name; title of paper, whether or not work is original; whether or not article has been published.
6. Five copies of a typewritten, double spaced paper must be submitted if the paper is to be considered for all awards.

7. Two copies of each manuscript must be submitted if not competing for the awards subject to the above instructions.
8. All audio and visual aids will be supplied by the Speakers' Supplies Committee. Please indicate what aids are needed for presentation of paper.

Read the March issue of A.J.M.T. for the Program Committee's report on the many other excellent speakers and activities.

Patricia Sallas, Chairman
Department of Pathology
Charity Hospital
New Orleans, La.

PIRATES ALLEY

Scene of outdoor art exhibit where New Orleans artists display paintings of New Orleans scenes.

Entertainment Committee Patter:

Few are the people who set foot on the sidewalks of New Orleans who do not seek to learn the location of the "Court of Two Sisters." To dance in a picturesque courtyard rich with romance, and to enjoy food prepared according to old recipes handed drown from one generation to another is a delight even to the most discriminating.

Patate, anxious to please our ASMT members, urged your Entertainment Committee to arrange for you an evening under the stars in the "Court of Two Sisters" at 615 Rue Royale, the very heart of the Vieux

Carré. You will revel in a true cosmopolitan atmosphere and relax with music in the softly lighted garden while praline mammies and pickaninny dancers endeavor to entertain you. Instead of soup to nuts, it will be Louisiana Gumbo to Cherry Jubilee.

In the last issue of our Journal we told you of our plans for an afternoon aboard the Steamer President. "Old Man River" will capture our attention for two hours and a half and give an opportunity to see our Port while we enjoy a get-together to discuss the scientific session prepared by your Program Committee.

Many other treats are in store for you and your friends and they will be listed in our next Journal.

Start planning now to be "WAY DOWN YONDER IN NEW ORLEANS" from June 12th to 17th when your Entertainment Committee will strive to convert your leisure moments into memorable occasions.

Anna J. Harnan
Entertainment Chairman
4532 S. Tonti Street
New Orleans 15, La.

Scientific Exhibit Patter:

It is not too early to reserve a booth for your scientific or state society exhibit. Remember it takes time to prepare the material and to make the arrangements for the exhibition. Probably many of you have some special or new technics. Therefore, why not share them with us? This is your opportunity.

The backwall of the booth will be medium blue bengaline flameproofed draped material. The background drapes are framed with fancy iron grille work so typically "New Orleans." The side dividers are also made of black wrought iron grille work. A table is furnished. Covering for the table is to be furnished by the exhibitor.

Please check the following when applying for exhibit space:

1. Size of booth.	All booths are 8 ft. x 6 ft.
2. Counter table.	Needed: Yes: No:
3. Chair.	Needed: Yes: No:
4. Electric outlet. One will be furnished if indicated.	Indicate if needed for display. Additional outlets $7.50.
5. Headboard sign.	11" x 28". One or two lines to read:

PLEASE PRINT

Name and address of applicant responsible for exhibit:_____

_____

_____
(Signature)

For further particulars or reservation, please write to:

Helen Beros, Chairman
4234 St. Claude Avenue
New Orleans 17, La.

Publicity Committee Patter:

The convention seal is a true emblem of what makes New Orleans "America's Most Interesting City."

Delicate lace ironwork furnishes a background for a miniature of the St. Louis Cathedral (oldest in the U. S.) and the fabulous Pontalba Buildings (America's first apartment houses) flanking the Place d'Armes (historic Jackson Square) and is artistically cut in silver and sky blue.

All official and personal mail of A.S.M.T. members and members of affiliate state and local societies should carry this decorative seal between now and June, 1955.

PRICE OF SEALS: Less than 500..... 1 cent each
Lots of 500..... $4.50
Lots of 750..... $6.75
Lots of 1000..... $8.00

You may obtain seals by sending your remittance and order to:
Olga C. Blanke, Chairman
804 Napoleon Avenue
New Orleans 15, La.

Registration Committee Patter:

Early registration saves your time and ours and spares the nerves. We who registered in advance for the Miami Beach Convention can recommend it highly. The advance registration forms will be available in the March-April issue of the A.S.M.T. Journal. Please use them.

Hazel Newton, Chairman
1137 Burgundy Street
New Orleans 16, La.

ANNOUNCEMENTS

The British Institute of Medical Laboratory Technology announces its Triennial Conference to be held at Nottingham University, August 29 to September 3, 1955. Members of the American Society of Medical Technologists who will be in Britain at that time are invited to attend. These Conferences have been held every third year since 1924, except during the war.

There will be papers read each morning (Monday through Friday), and "during the afternoons and evenings various social and scientific events will be arranged, including a Trade Show of modern laboratory equipment and apparatus by various trade houses. Arrangements are also being made for at least one symposium on a subject of general interest."

Those who expect to attend should notify the Conference Secretary as soon as possible.

Write: G. Whiting, Esq., F.I.M.L.T.
The Priory
Thurgarton
Nottingham, England

The University of Texas Post Graduate School of Medicine announces a seventeen-hour course in radioisotopic techniques for medical technologists on March 3, 4, and 5, 1955 at the M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Fee $10.00. The course is designed for trained medical technologists to acquaint them with the basic techniques. Atomic Energy Commission training films, together with lectures and demonstrations, will be featured. Emphasis will be placed on the details of technique with current clinical use. Various instruments now used will be available for inspection, with limited use by the registrants. For further information write to the University of Texas Post Graduate School of Medicine, Texas Medical Center, Houston 25, Texas.